

NEW REACTIONS OF CEPH-3-EMS

A THESIS SUBMITTED BY
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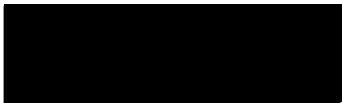
DOCTOR OF PHILOSOPHY

Dundee Institute of Technology

November 1989

DECLARATION

I hereby declare that the work presented in this thesis was carried out by me at Dundee Institute of Technology, Dundee and at Beecham Pharmaceuticals, Brockham Park, Surrey except where due acknowledgement is made, and has not been submitted by me for any other Degree.

Signed 

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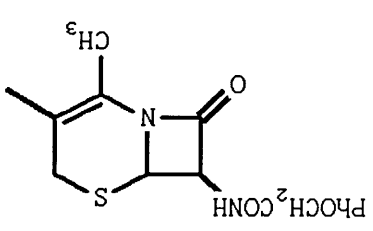
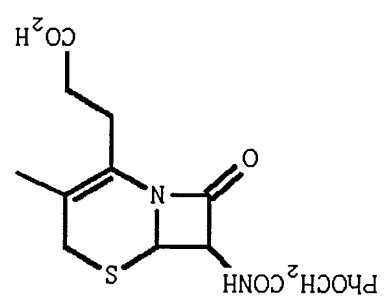
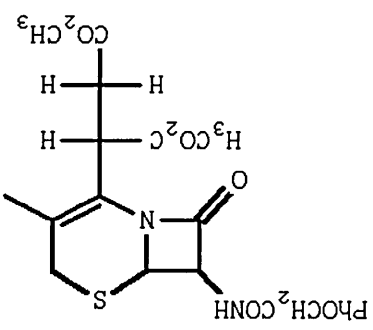
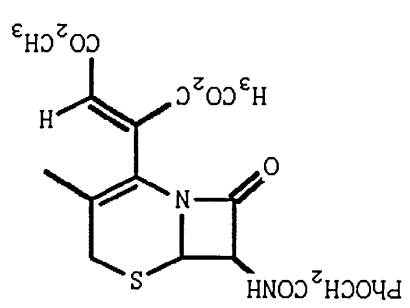
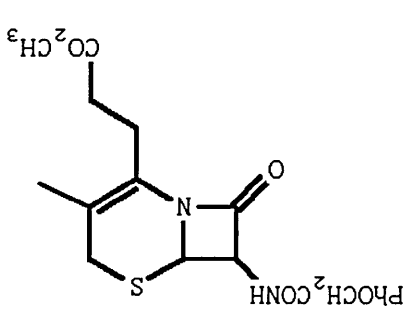
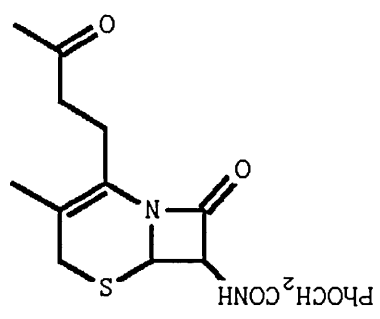
ABSTRACT

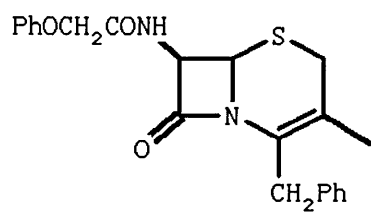
A 3-step process for the conversion of C-4 disubstituted ceph-2-ems into ceph-3-ems, where the original C-4 carboxyl group is replaced by a substituted ethyl group, is described. A range of novel compounds (A-D) are produced. Ester (B) is converted into carboxylic acid (E).

The regiospecificity of alkylation reactions of ceph-3-em sulphides, α -sulphoxides and β -sulphoxides is investigated for a variety of bases and electrophiles. C-4 disubstituted ceph-2-ems thus obtained are converted into the novel β -lactams (F & G).

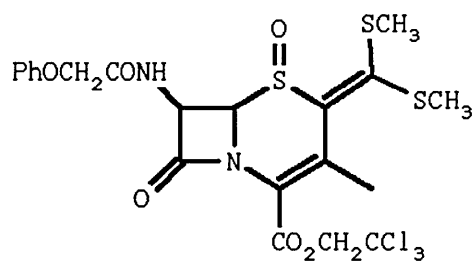
A novel class of ceph-3-ems bearing C-2 exocyclic double bond systems containing two heteroatoms is described (H-J). Ester (H) is converted into acid (L).

None of the new β -lactams obtained exhibited any significant biological activity.

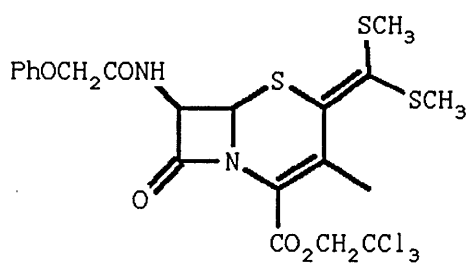




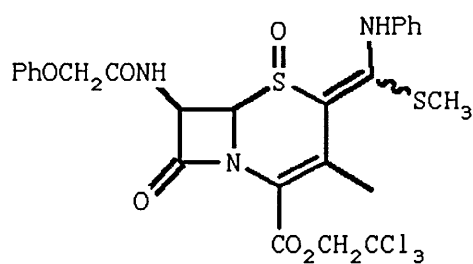
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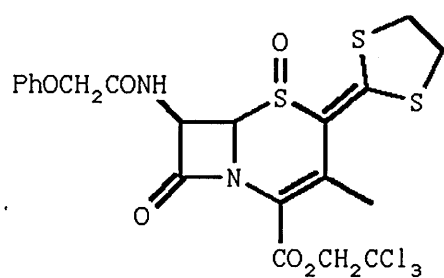
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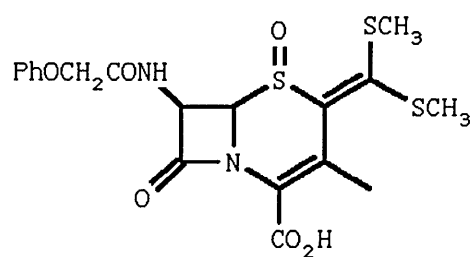
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FOREWORD

Bracketed Arabic numerals in the text refer to the diagrams of the formulae and the Arabic superscripts indicate references. The following abbreviations have been used in the text.

Ac	acetyl group CH_3CO
BOC	tertiary butyloxy carbonyl group
Bu ^t	tertiary butyl group
BSA	bis(trimethylsilyl)acetamide
<i>m</i> -CPBA	<i>meta</i> -chloroperoxybenzoic acid
δ	parts per million
DBN	1,5-diazabicyclo[4.3.0]non-5-ene
DBO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DMAD	dimethyl acetylenedicarboxylate
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulphoxide
DPM	diphenylmethyl group
Et	ethyl group
G	phenylacetamido
ir	infrared
LCIA	lithium cyclohexylisopropylamide
LDA	lithium diisopropylamide
Me	methyl group
MIC	minimum inhibitory concentration

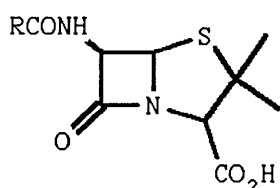
Ms	methanesulphonyl group
mp	melting point
NCS	N-chlorosuccinimide
NBS	N-bromosuccinimide
NOE	nuclear Overhauser enhancement
PBP	penicillin binding protein
Ph	phenyl group
PMB	<i>para</i> -methoxybenzyl group
pmr	proton magnetic resonance
PNB	<i>para</i> -nitrobenzyl group
PPTS	pyridinium <i>para</i> -toluene sulphonate
PTSA	<i>para</i> -toluenesulphonic acid
TCE	trichloroethyl group
THF	tetrahydrofuran
tlc	thin layer chromatography
Ts	<i>para</i> -toluenesulphonyl group
uv	ultraviolet
V	phenoxyacetamido

INTRODUCTION

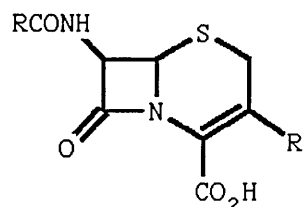
1.1) INTRODUCTION

Perlman¹ has stated that "micro-organisms can do everything, micro-organisms are wiser than chemists", and the vast array of naturally occurring antibiotics isolated from micro-organisms (currently more than 6000²) bears witness to this statement. In clinical use however, only a few basic structures predominate, with the β -lactam antibiotics accounting for around 60% of the world market³.

The β -lactam story now extends over 60 years from the initial discovery by Fleming⁴ of the antimicrobial activity of penicillin (1). The introduction of penicillin G (1; R=PhCH₂) into clinical use in the late 1940s was followed by the discovery of the cephalosporins (2) in 1955⁵. Chemical synthesis has

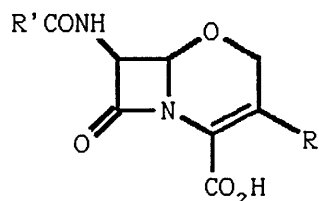


(1)

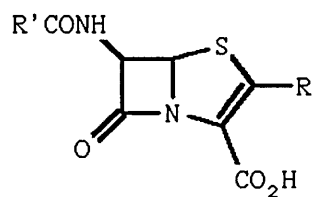


(2)

afforded penicillins and cephalosporins with a wide variety of amide substituents, and in the case of the cephalosporins, many C-3' substituents have been developed. Totally synthetic β -lactams have also been prepared, the most successful examples being the oxacephems⁶ (3) and penems⁷ (4).

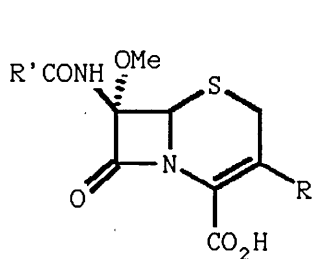


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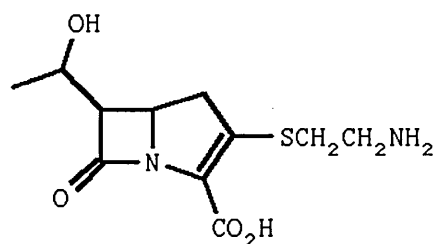


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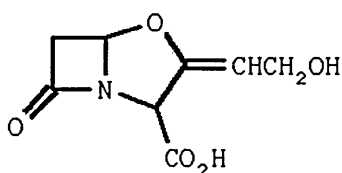
As suggested by Perlman, micro-organisms have also yielded a large number of biologically active β -lactams. Thus, *Streptomyces* species have been found to produce cephamycins⁸ (5), carbapenems⁹ (6) and clavulanic acid¹⁰ (7). Other β -lactam antibiotics, including members of



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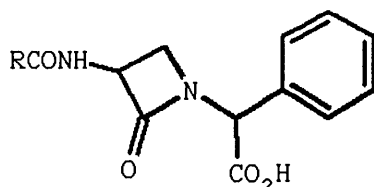


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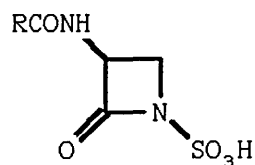


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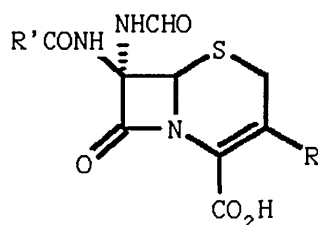
the nocardicin¹¹ (8), monobactam¹² (9) and chitinovorin¹³ (10) families also occur naturally.



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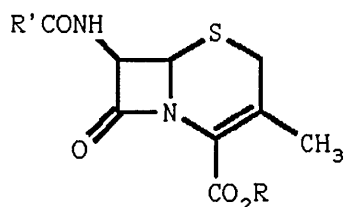


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The research programme described in this thesis involves modifications to the dihydrothiazine ring of ceph-3-ems (11) without affecting the β -lactam carbonyl



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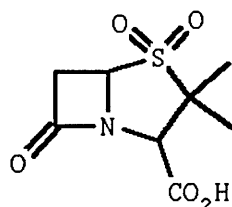
moiety. In order to put this work in context, recent literature pertinent to this area is reviewed. Several reviews¹⁴⁻¹⁶ have covered the period prior to 1980 and this introduction will therefore consider publications since then, however publications before 1980 will be referred to where appropriate. No attempt has been made to include the vast number of side-chains coupled to the amino group at the C-7 position of the cephem, nor has the direct introduction of substituents leading to

3-(substituted)methyl cephems been considered unless they are of direct relevance.

1.2) REACTIONS AT SULPHUR

1.2.1) Oxidation

Oxidation of the dihydrothiazine ring sulphur in cephalosporins and the corresponding penicillin sulphur to sulfoxides and sulphones is of great synthetic value. Uses of oxidised cephalosporins and penicillins include the acid-catalysed ring expansion of penam sulfoxides into deacetoxycephalosporins¹⁷; as intermediates for the halogenation and further functionalisation of the C-3' position of deacetoxycephalosporins^{18,19}, and in the preparation of novel antibiotics and β -lactamase inhibitors such as sulbactam (12)²⁰. Additionally, Merck researchers have reported²¹



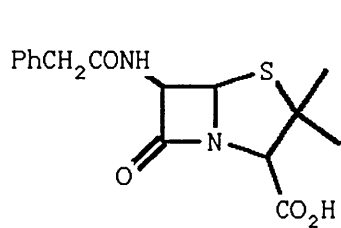
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that 7 α -substituted cephalosporin sulphones with either C-4 ester or amide groups are potent inhibitors of human leukocyte elastase, an enzyme which has a degradative effect on lung elastin.

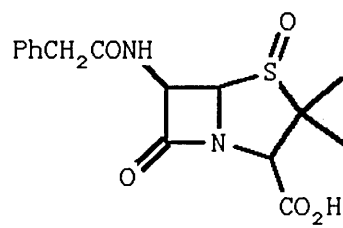
As a consequence of these continued uses for oxidised β -lactams, a wide variety of methods claiming higher yielding, or more facile, routes to penicillin and

cephalosporin sulfoxides and sulphones have emerged over recent years²².

It is, however, generally accepted that oxidation of the dihydrothiazine (thiazolidine) ring sulphur in cephalosporins (penicillins) results in compounds which have decreased antibacterial activity compared to the parent sulphide. Attempts to rationalise the reduced activity of penicillin sulfoxides on the basis of X-ray data have been made²³. Comparison of the molecular parameters of penicillin G (13) and its 1 β -sulphoxide (14) showed little difference in the N4-C7 bond length



(13)



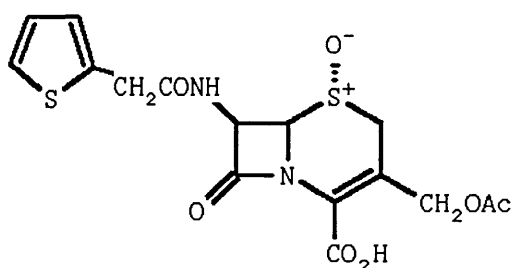
(14)

or in the planarity of the β -lactam nitrogen atom, both parameters previously used to provide rough estimates of biological activity^{24, 25}. The molecular structures of (13) and (14) did differ, however, in the conformation of the amide side chain; the conformation of the C-3 carboxylic acid, and the puckering of the thiazolidine ring (the latter two effects being inter-related).

It was reported that in the case of (14), as has previously been observed^{26, 27}, the carboxyl group adopts the 3 α -equatorial conformation whereas the parent sulphide adopts the 3 α -axial conformation. Computer simulation of the theoretical 3 α -axial carboxyl

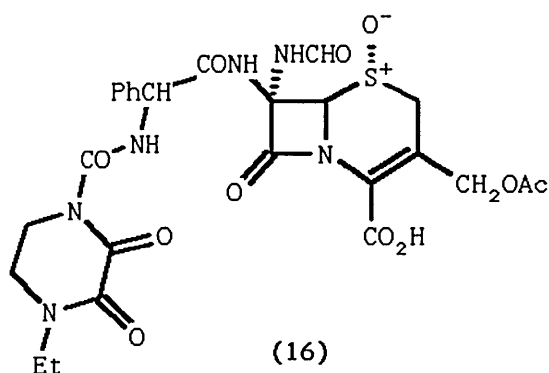
sulphoxide resulted in very close proximity between the C-7 amide nitrogen and the sulphoxide oxygen. This spatial proximity was interpreted as an indication of the energetic unfavourability of this conformation. Therefore, although the data presented does not definitively prove the reasons for the reduced activity of penicillin sulphoxides, the proposed conformation of the thiazolidine ring may interfere with the ability of these molecules to bind with penicillin-binding proteins. This inability to bind will decrease the biological activity of the sulphoxides compared to the sulphides which can bind to the PBPs.

In 1976 de Koning and co-workers reported²⁸ that a variety of cephem 1 α -sulphoxides (for example (15)) were



(15)

biologically active, whereas the isomeric β -sulphoxide retained no activity after oxidation of the parent sulphide. The magnitude and spectrum of activity of the α -sulphoxide was, however, reduced. Subsequently, Beecham workers have reported²⁹ that 7 α -formamidocephem 1 α -sulphoxide (16) has considerably enhanced Gram



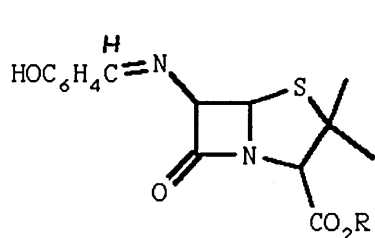
negative activity with respect to the 1 β -sulphoxide.

Due to the conformation adopted by the dihydrothiazine ring, the α -face is sterically less hindered and oxidation would therefore be expected to occur on the α -face. It has been observed³⁰, however, that in penicillins and cephalosporins bearing a C-6 (C-7) amide side-chain, the 1 β -sulphoxide is formed almost exclusively. This has been rationalised³⁰ by the concept of 'reagent approach control' whereby the 6- β (7- β) amide proton forms a hydrogen bond with the peracid oxidising agent, resulting in oxidation on the β face. Three general routes to overcome this problem have been utilised, viz:

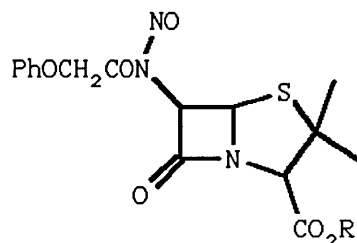
- the use of a non-peracid oxidant which will not form a hydrogen bond with the amide side chain eg: ozone³¹.
- reagents which initially form a β -sulphonium chloride which hydrolyses with inversion of configuration eg: iodobenzene dichloride³², N,N-dichlorourethane³³, N-monochlorourethane³⁴.

- protection of the amide proton by a group which can be easily cleaved after peracid oxidation.

Protective routes involving the Schiff's base²⁸ (17) and N-nitroso compound³⁵ (18) have also proved

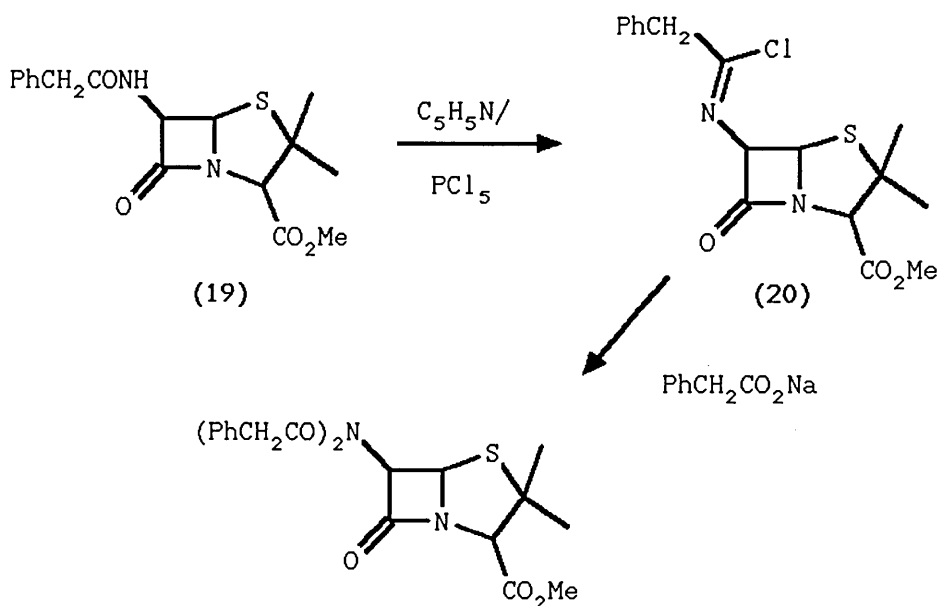


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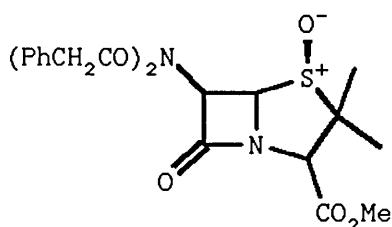
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effective. These early routes to penicillin and cephalosporin α -sulfoxides have been reviewed elsewhere³⁶. In continuing studies on the oxidation of sulphur in bicyclic β -lactams, Micetich *et al*³⁷⁻³⁹ have reported methods for the synthesis of α -sulfoxides. Reaction of the 6-acylamino penicillin (19) with pyridine/ PCl_5 gave the 6-chloroimine (20) which reacted

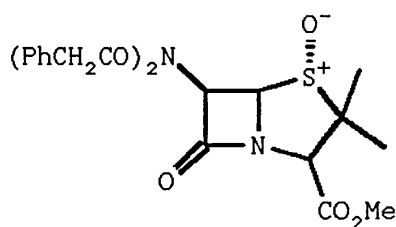


(21)

with sodium phenylacetate to give the 6-(diacylamino) penicillin (**21**) in an overall yield of 36%³⁷. Oxidation of the (diacylamino)penicillin with *m*-CPBA resulted in a mixture of the β - and α -sulphoxides (**22**) and (**23**), the

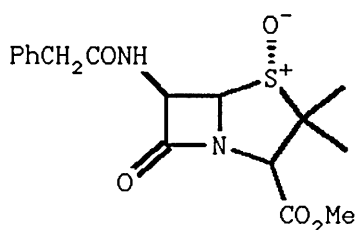


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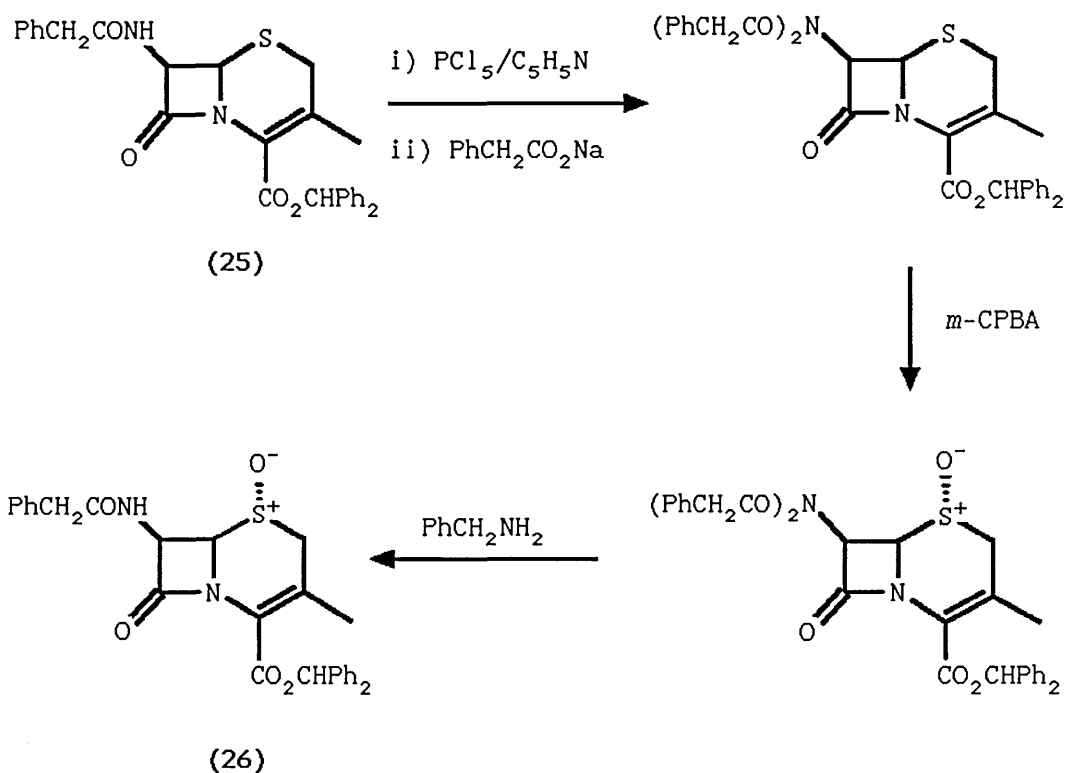
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ratio of (**22**) to (**23**) being dependent on solvent, C-3 ester group and reaction temperature. Optimisation of these variables achieved almost exclusive formation of α -sulphoxide (**23**). Removal of the 6-acylamino protecting group was effected using zinc/ammonium acetate to give the α -sulphoxide (**24**), though only in 14% overall yield from (**19**).

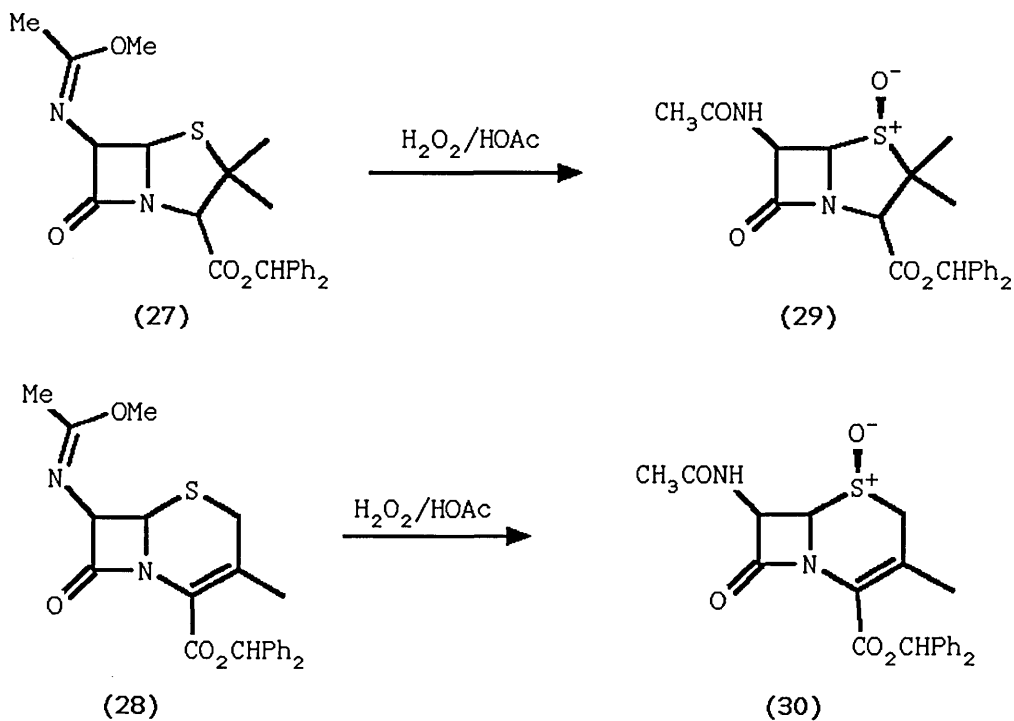


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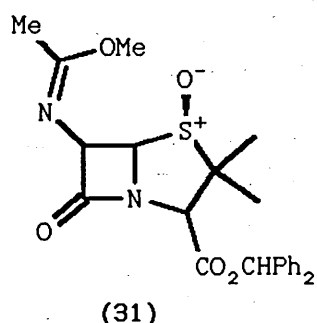
This methodology has also been extended to the cephem series³⁸ where cephem (**25**) is converted into the α -sulphoxide (**26**) in 35% overall yield. The same



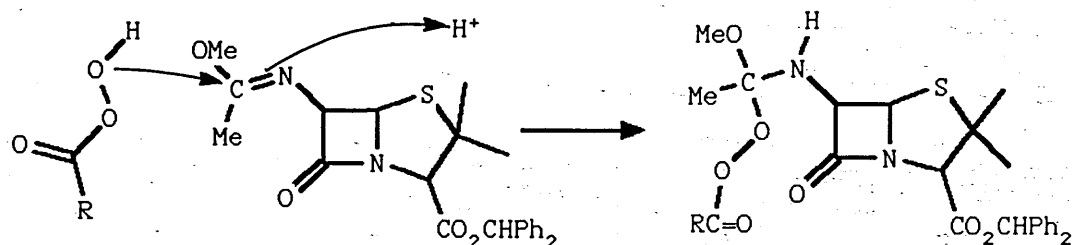
authors also proposed³⁹ that oxidation of penicillin or cephalosporin 6-(7)-iminoethers would result in formation of the α -sulfoxide for the aforementioned reasons. Oxidation of penam (27) or cephem (28) with



$\text{H}_2\text{O}_2/\text{HOAc}^{40}$ resulted in formation of the β -sulphoxides (29) and (30) respectively, with concomitant cleavage of the iminoether group. When (27) was oxidised with *m*-CPBA, the corresponding β -sulphoxide iminoether (31) was obtained. Formation of the β -sulphoxides (29) and



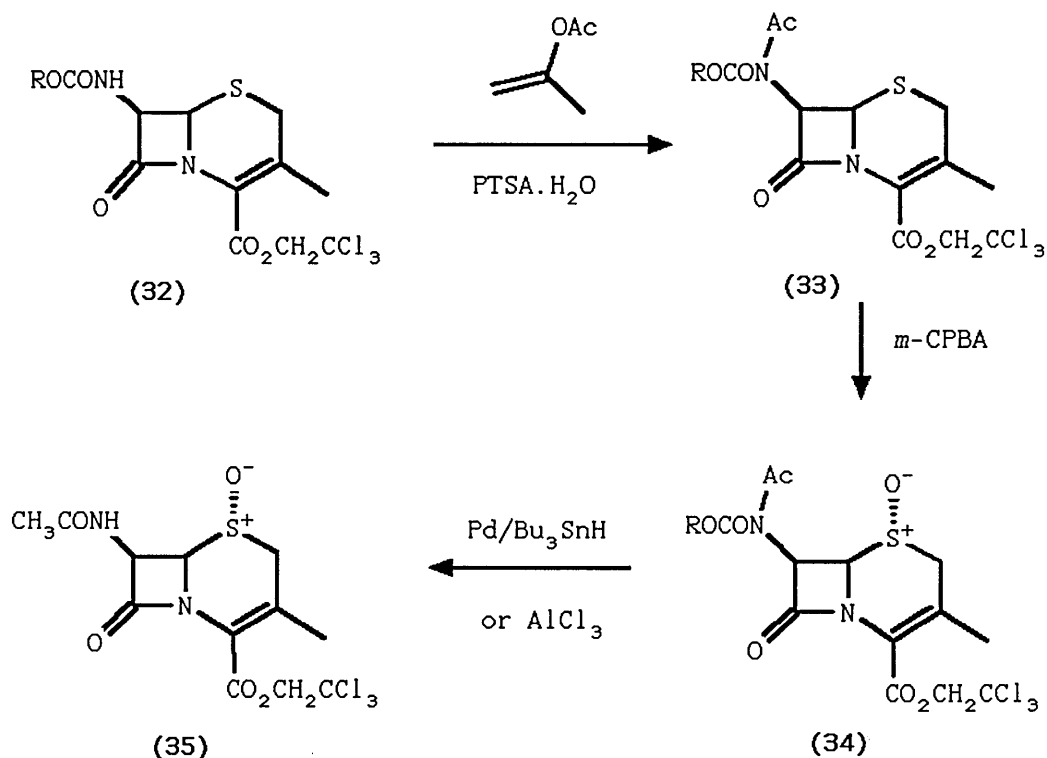
(30) can be rationalised by initial addition of the oxidising agent to the iminoether, thereby allowing steric control of the oxidation to produce the sterically more hindered β -isomer as shown in Scheme 1.



Scheme 1

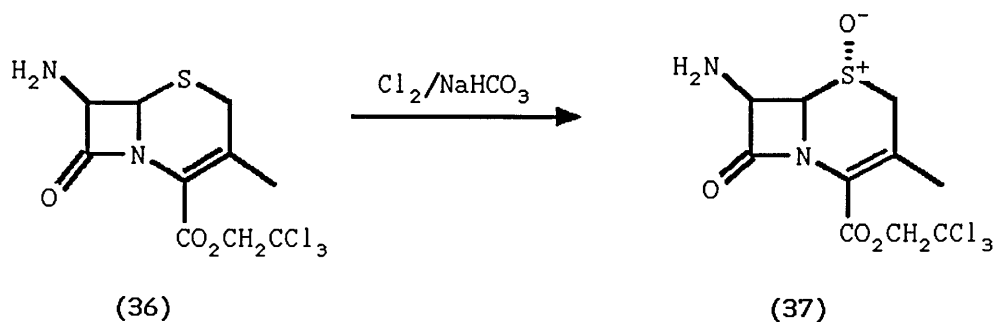
Spry and co-workers⁴¹ have also explored the use of cephem C-7 imides in the synthesis of α -sulphoxides. Reaction of the C-7 carbamate (32) with isopropenyl acetate in the presence of PTSA monohydrate resulted in formation of the N-acetyl derivative (33). Oxidation of (33) with *m*-CPBA afforded α -sulphoxide (34) in high yield, which was subsequently de-protected with AlCl_3 or

Pd/Bu₃SnH to give the 7-amidocephem (35). These authors



R = CH₃, PhCH₂ or allyl

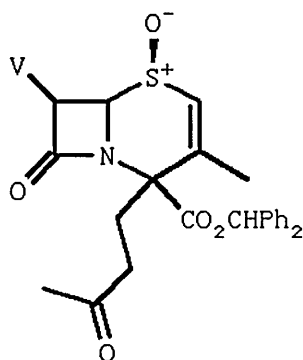
also report that oxidation of 7-aminocephems (36) with Cl₂/NaHCO₃⁴² results in good yield of the α-sulphoxide (37).



1.2.2) De-oxygenation

De-oxygenation of penicillin and cephalosporin sulfoxides to the respective sulphides normally requires prior activation of the sulfoxide, followed by de-oxygenation with a reducing agent⁴³. Micetich⁴⁴ has subsequently found that P₂S₅/pyridine results in

de-oxygenation of β -lactam sulphoxides. The failure of simple reducing agents to bring about de-oxygenation has been attributed⁴³ to electronic effects in the ceph-3-em sulphoxide. In ceph-2-em sulphoxides (38), it has been suggested⁴⁵ that similar electronic effects do not exist since de-oxygenation readily occurs under mild conditions using zinc in DMF/glacial acetic acid. (See Section 2.1.5 for further discussion.)

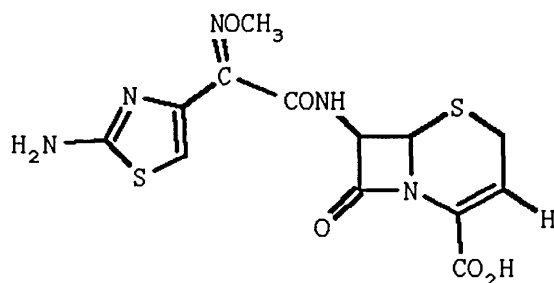


(38)

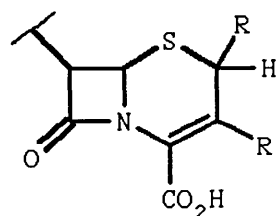
1.3) REACTIONS AT C-2

Although a wide variety of C-2 substituted cephalosporins have been prepared⁴⁶, substitution at this position has resulted in decrease or loss of biological activity with the exception of 2-alkyl and 2-alkylidene derivatives.

A study of 2-alkyl derivatives of ceftizoxime (39)⁴⁷ has shown that the stereochemistry of the 2-substituent has a marked effect on biological activity. When the



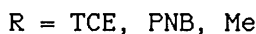
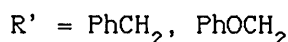
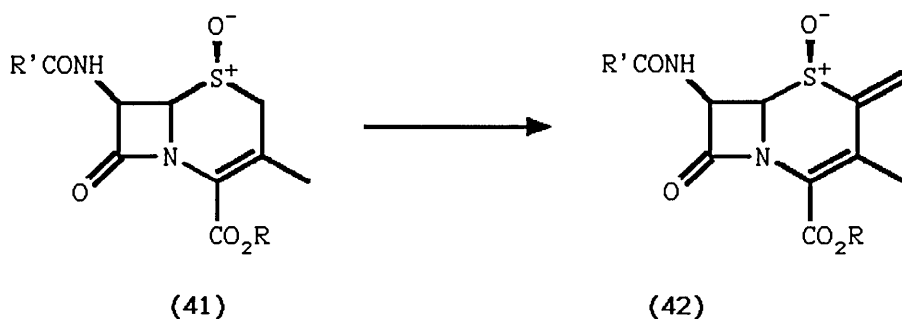
(39)



(40)

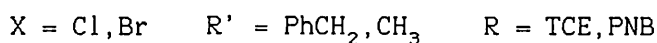
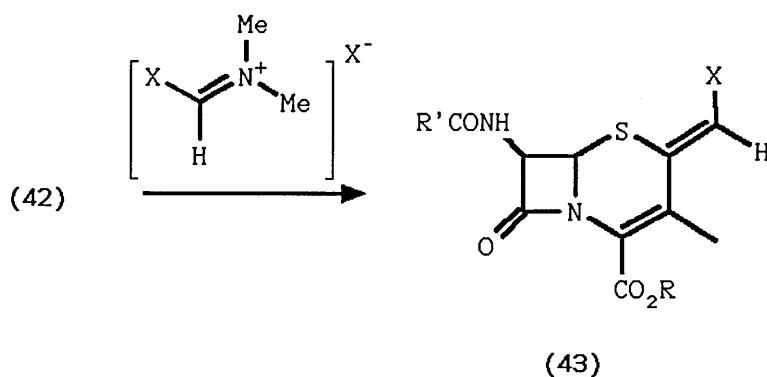
2,3-dimethyl analogue (40; $R=\alpha\text{-CH}_3$, $R'=\text{CH}_3$) was synthesised (no preparative details are given), a marked decrease in activity was observed compared to the 3-methyl derivative (40; $R=\text{H}$, $R'=\text{CH}_3$). When the 3-methyl group was not present, however, the 2-methyl analogue (40; $R=\alpha\text{-CH}_3$, $R'=\text{H}$) was found to have enhanced activity over the 2,3-dimethyl derivative. The 2 α -methyl analogue (40; $R=\alpha\text{-CH}_3$, $R'=\text{H}$) was considerably more active against a range of Gram positive and Gram negative bacteria, and only slightly less active than the parent molecule, ceftizoxime (39). Subsequently, synthesis of 2 α -methyl and 2 β -methyl derivatives of cephalosporins with differing C-7 and C-3' substituents has been performed.⁴⁸ This has shown that, in general, the 2 β -methyl isomers have greater antibacterial activity than the corresponding 2 α -methyl isomers.

The introduction of exocyclic double bonds at the 2-position under a variety of conditions has been reported. C-2 Exomethylene cephalosporins (42) have previously been synthesised by a Mannich reaction of the corresponding β -sulphoxide (41)^{49,50}. Further work to



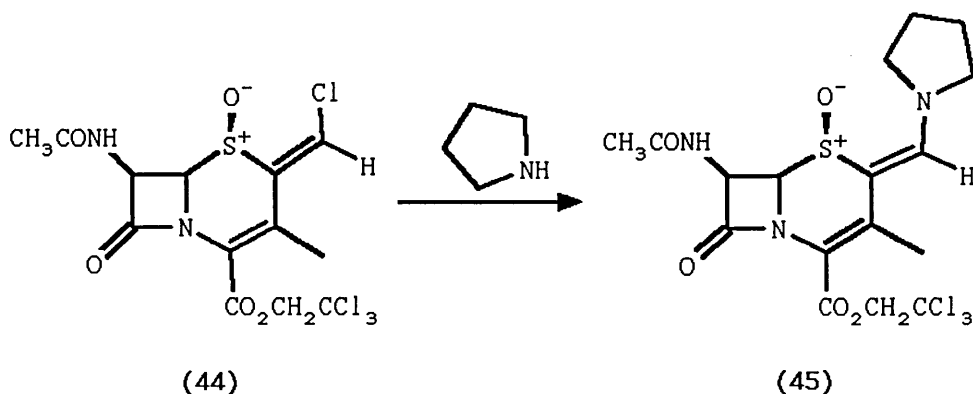
establish the steric limitations of this reaction has shown⁵⁰ that the β -sulfoxide and sulphone undergo the Mannich reaction, whereas the α -sulfoxide does not react. Increasing the reaction temperature with the α -sulfoxide resulted solely in the formation of degradation products. Initial attack by the Mannich reagent occurs on the sterically less hindered α -face, hence in the α -sulfoxide the Mannich base cannot be formed due to steric hindrance.

The 2-exomethylene cephem has been reacted with Vilsmeier's reagent^{51, 52} to yield C-2 vinylhalocephems (43). The reaction was found to be stereospecific, as

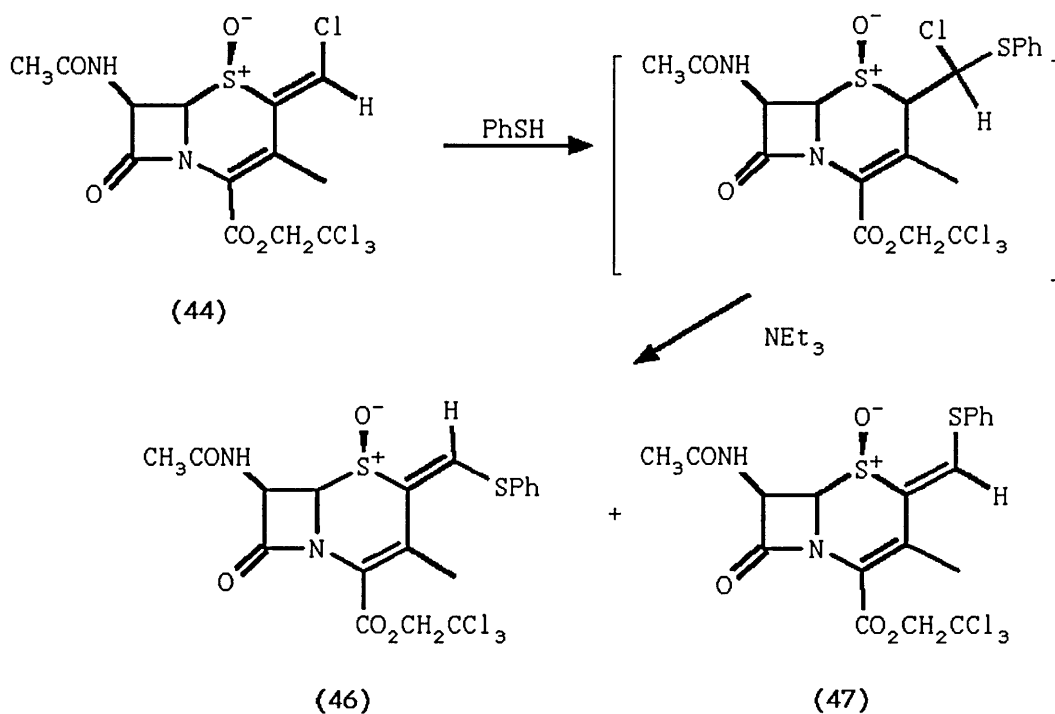


evidenced by an NOE between the C-2 vinyl proton and the C-3 methyl group. These vinylhalocephems have also been

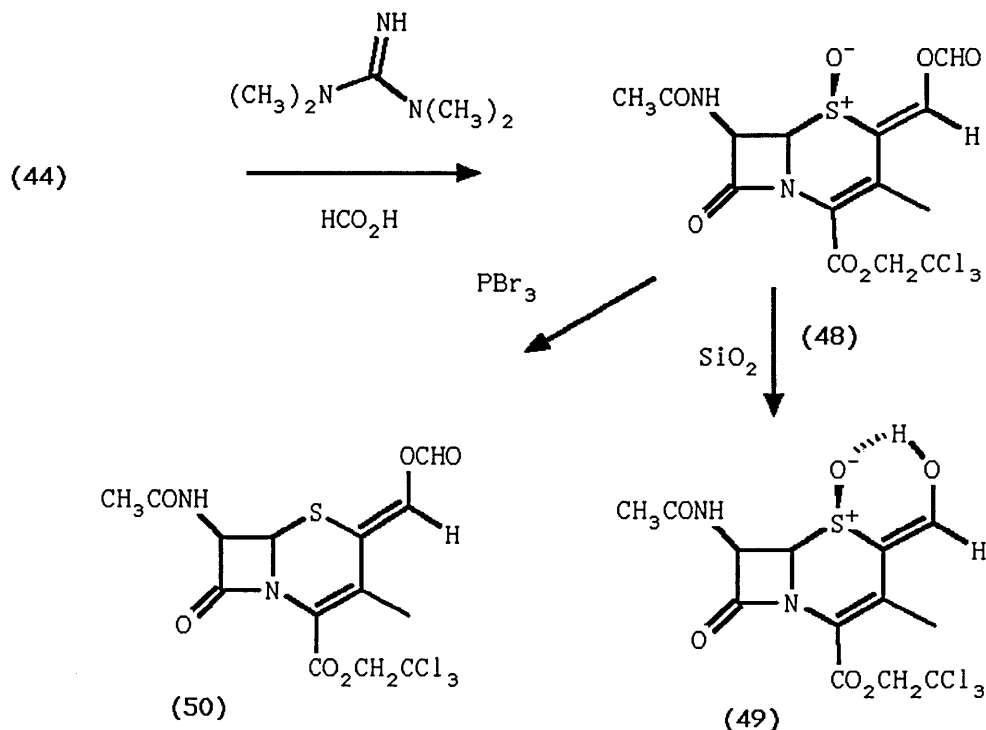
prepared⁵³ by reaction of the 2-exomethylene sulphoxide (42) with dimethyl immonium halides in a Pummerer-type reaction. C-2 vinylhalocephems have been oxidised and reacted⁵¹ with primary and secondary amines to form stable enamines (45).



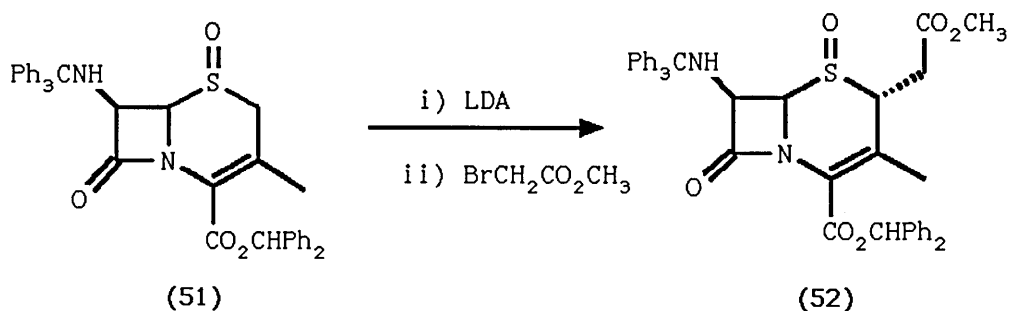
Reaction of sulphoxide (44) with thiols gave addition products which were unstable to column chromatography, however elimination of HCl with triethylamine resulted in a mixture of the E- and Z-vinylsulphides (46) and (47). Further compounds containing C-2 exocyclic double



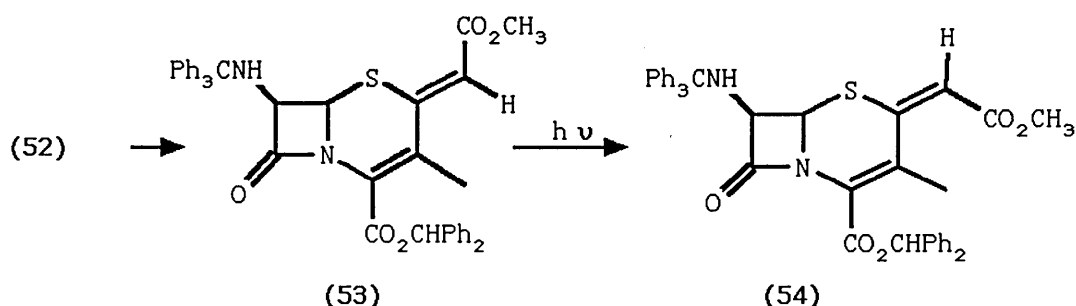
bonds were obtained when sulphoxide (44) was reacted with tetramethylguanidinium formate to give the vinyl formate (48) which was unstable to silica gel, resulting in the H-bonded enol (49). Reduction of (48) with PBr₃ afforded sulphide (50). Compounds containing C-2



exocyclic double bonds prepared from routes other than via C-2 exomethylene cephem (42) have also been reported⁵⁴. Reaction of sulphoxide (51) with LDA generates a C-2 carbanion which is trapped with methyl bromoacetate to give the C-2 ester (52), which has α -stereochemistry at the 2-position. Sulphoxide (52)

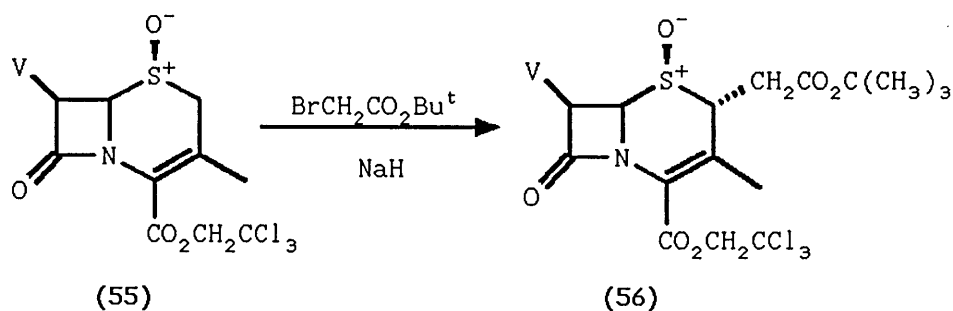


undergoes a Pummerer rearrangement with trifluoroacetic anhydride/acetic anhydride. Addition of lutidine forms the exocyclic double bond system (53). (Z)-Stereochemistry was assigned on the basis of a large NOE between the vinyl proton and the C-3 methyl group, however irradiation of this pure isomer resulted in a 35:65 mixture of the (Z)- and (E)-isomers (53) and (54).



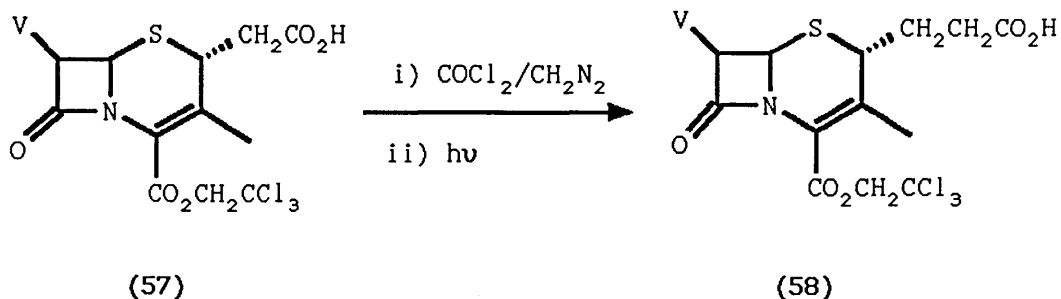
All of the (Z)-isomers prepared, with differing C-7 substituents were found to have some Gram positive activity, but little against Gram negative organisms. The (E)-isomer (54) had enhanced Gram negative activity (in some cases better than cephalothin) and good Gram positive activity.

Spry has recently reported⁵⁵ a similar reaction between cephem sulfoxides, *t*-butyl bromoacetate and sodium hydride to produce (56). De-oxygenation of

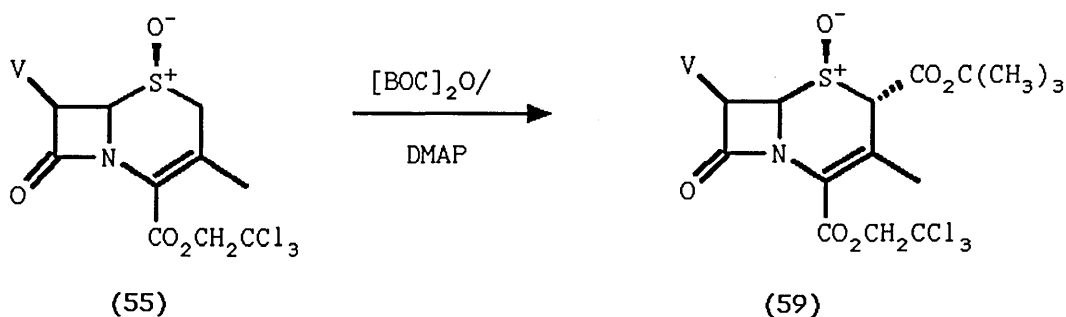


sulfoxide (56) followed by de-protection of the *t*-butyl group with TFA gave the corresponding C-2 carboxylic

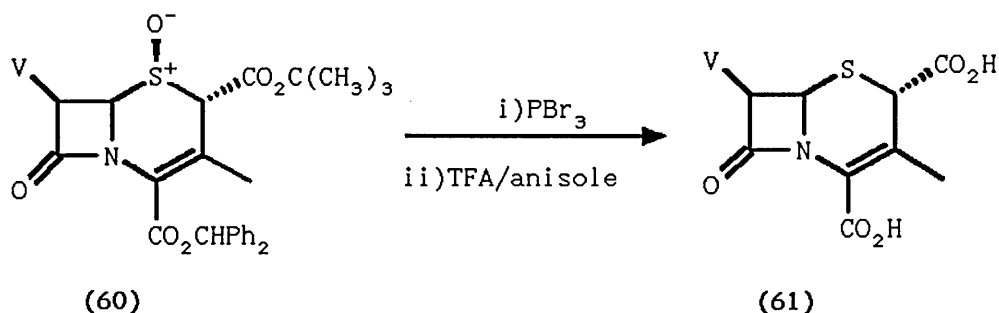
acid (57) which was biologically inactive. This acid was converted to homologue (58) via an Arndt-Eistert reaction.



The use of BOC anhydride/*N,N*-dimethylaminopyridine to protect the N-7 proton in cephem sulphides and α -sulphoxides is well known⁴¹. In the case of β -sulphoxides and sulphones however, the same reagents do not produce the N-BOC derivatives, rather C-2 carboxylation occurs to produce (59). De-esterification

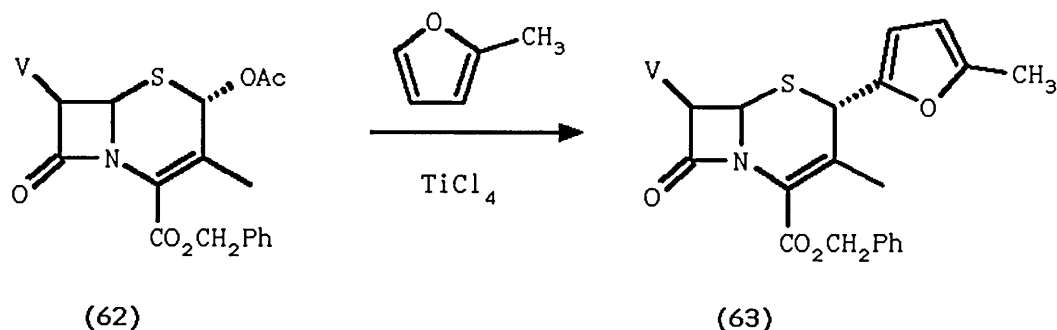


of (59) results in the C-2 carboxylic acid which spontaneously decarboxylates to reform the starting material (55). De-oxygenation of sulphoxide (60) to the corresponding sulphide, followed by de-esterification resulted in the stable 2 α -carboxylic acid (61) which showed only poor antibacterial activity. The lack of



activity found in compounds (57) and (61) is proposed as being a consequence of the altered stereochemistry of the 2-position⁵⁵.

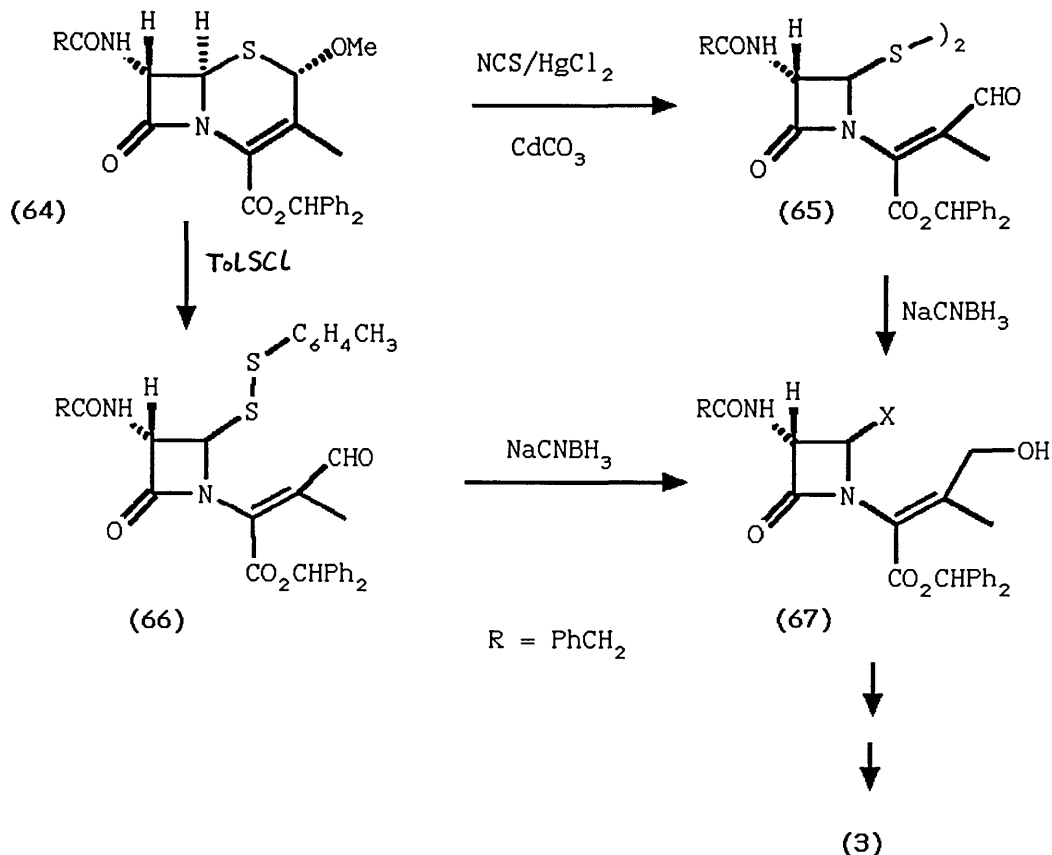
A wide variety of C-2 substituted cephems have been synthesised⁵⁶ *via* the replacement of C-2 methoxy and acetoxy substituents by nucleophiles in the presence of a Lewis acid catalyst (eg: TiCl_4 , AlCl_3). When 2-acetoxycephem (62) was reacted with 2-methylfuran at -25°C for 4 hours in the presence of TiCl_4 , the 2-furylcephem (63) was obtained in high yield. Other



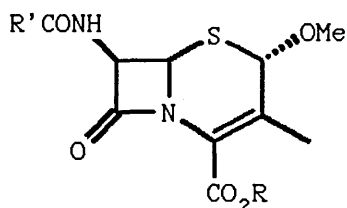
groups added at the 2-position in this manner include 2-furyl, 5-methyl-2-thienyl, carboxyethylthio, butylthio and allyloxy.

2-Alkoxycephems have also been used as precursors for the preparation of 1-oxacephems⁵⁷. The 2 α -methoxycephem (64) is ring-opened with either $\text{NCS}/\text{HgCl}_2/\text{CdCO}_3$ in water to give disulphide (65), or with *p*-toluenesulphonyl

chloride to give (66). Reduction of either aldehyde with sodium cyanoborohydride results in the corresponding alcohol (67) which undergoes ring-closure with mercuric trifluoroacetate followed by epimerisation at C-7 with LiOMe and *t*-butyl hypochlorite.



Due to this increased interest in the reactions of 2-alkoxycephems, coupled with the lengthy and/or low yielding existing routes to these compounds^{4,6}, several more facile routes to 2-alkoxycephems have recently been reported^{5,8-62}. Oxidation of cepems in a 3:1 mixture of MeOH/THF at +1.1V in the presence of tetraethylammonium tosylate resulted in formation of the 2-methoxy derivative (68)^{5,8} in yields (40-70%) which depended on the ester protecting group. Replacement of methanol by



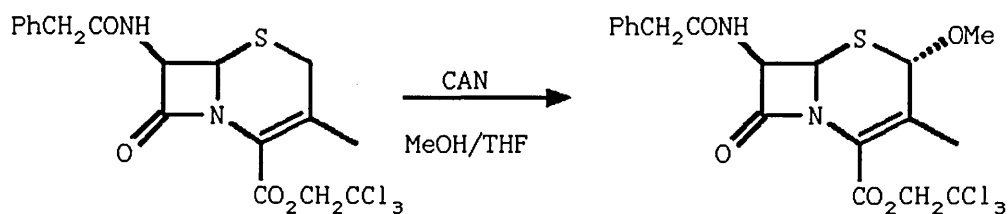
(68)

R = DPM, PNB, Bu^t

R' = PhOCH₂

ethanol, propan-2-ol or benzyl alcohol resulted in the 2-ethoxy, isopropoxy and benzyloxy derivatives respectively.

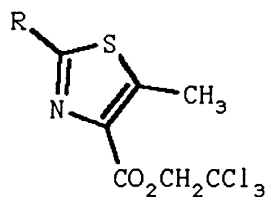
In a similar manner, reaction of cephem (69) with ceric ammonium nitrate (CAN) in methanolic THF gave the 2-methoxycephem (70) in 50% yield⁵⁹. It is interesting



(69)

(70)

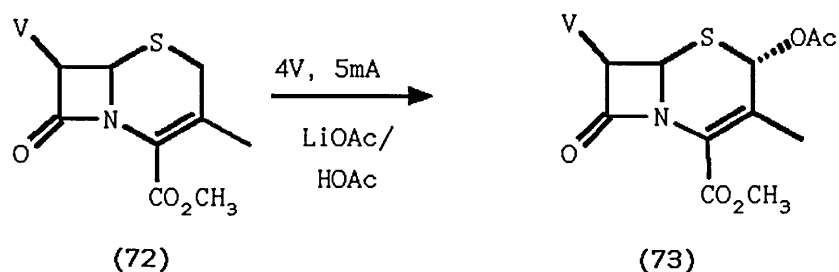
to note that previous reports⁶⁰ of the reaction of cephem (69) with CAN under acidic conditions resulted in a variety of thiazole products of type (71) stemming from β -lactam cleavage. In the more recent work⁵⁹ the



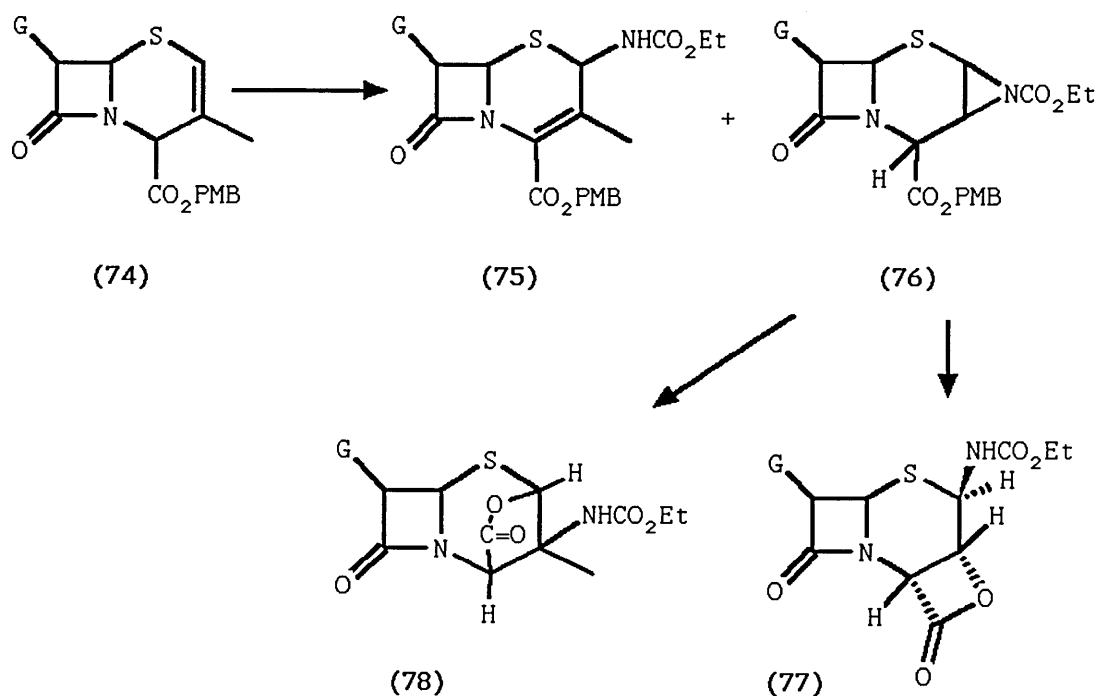
(71)

milder reaction conditions presumably minimise cleavage of the β -lactam system allowing reaction to occur at the 2-position.

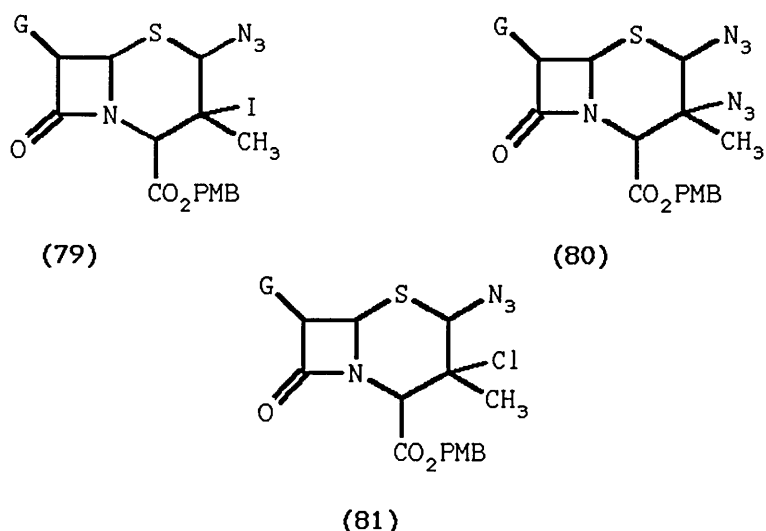
Electrolytic methods have also been used⁶¹ to produce 2-alkoxy and 2-acyloxy cephem derivatives in two-phase systems. For example, electrolysis of cephem (72) in acetic acid/lithium acetate solution at 4V (5mA) gave the 2-acetoxycephem (73) in high yield.



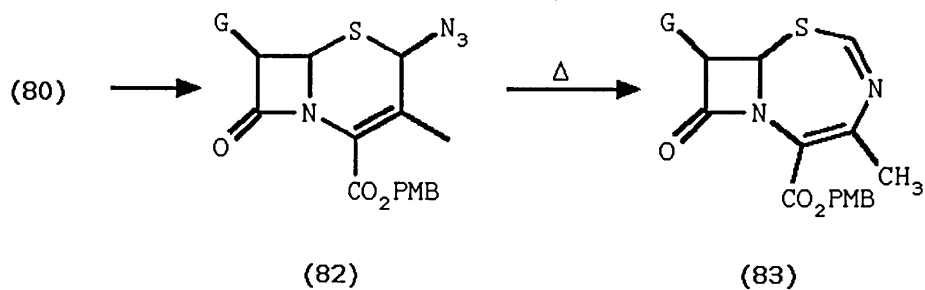
In a series of papers, Hoshide *et al* have described the reactions of ceph-2-em (74) with ethoxycarbonyl nitrene⁶³. The 2-ethoxycarbonylaminoceph-3-em (75) and aziridine (76) are produced. (76) reacted further to form *exo*- and *endo*-lactones (77) and (78).

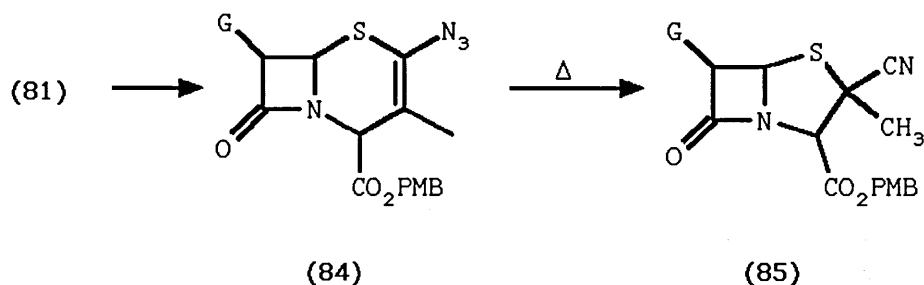


When ceph-2-em (74) was reacted with iodine monochloride and sodium azide^{6,4} the reaction products were found to be dependent on the solvent used. In acetonitrile or ethyl acetate the iodoazide (79) was obtained, whereas in nitromethane or carbon tetrachloride, diazide (80) was produced and in dioxane or dichloromethane, the chloroazide (81) was isolated. Treatment of (80) with



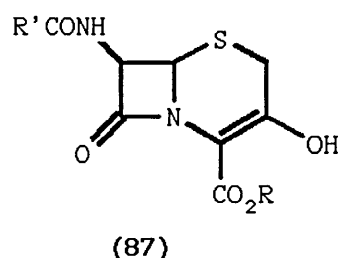
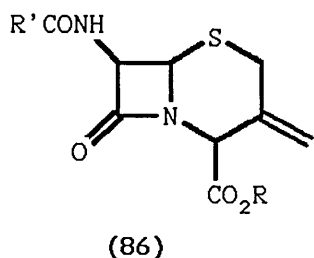
DBO gave^{6,5} the 2-azidoceph-3-em (82) which after reflux in CCl_4 afforded the thiadiazabicyclononadiene (83). DBO treatment of chloroazide (81) produced intermediate 2-azidoceph-2-em (84) which afforded penam (85) after reflux in benzene.





1.4) REACTIONS AT C-3

Historically, 3-substituted cephem derivatives have been produced from the 3-exomethylenecepham (86)⁶⁶, either directly or after ozonolysis to the 3-hydroxycephem (87)⁶⁷ which can then be further reacted to afford cephems with alternative 3-substituents^{68, 19}.

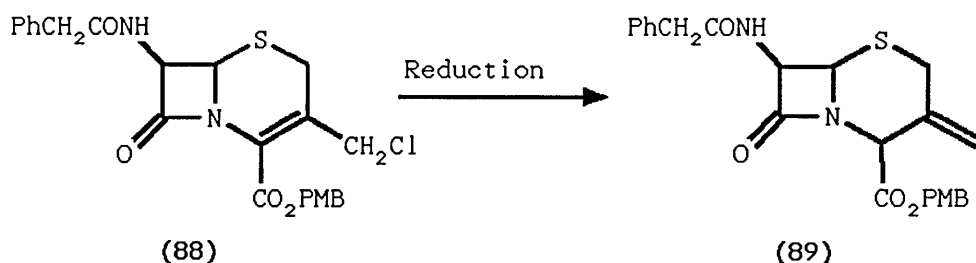


The 3-substituent has a marked effect on the β -lactam carbonyl system - electronegative substituents facilitate cleavage of the molecule resulting from nucleophilic attack at the β -lactam carbonyl. The C-3 group also affects antimicrobial and pharmacokinetic properties of the molecule such as enteral absorption. The following sections detail newer methods for the synthesis of cephalosporins directly substituted at the 3-position of the dihydrothiazine ring; novel reactions at the C-3' position are not discussed here.

1.4.1) C-3 Exomethylenecephams

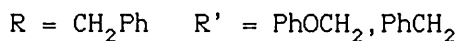
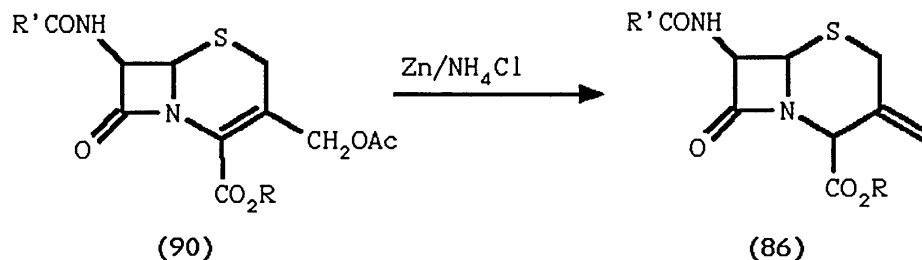
C-3 Exomethylenecephams have previously been prepared either from chemical or electrochemical reduction of C-3 substituted cepheids, or from penicillin sulfoxides (for economic reasons) *via* chemical or photochemical syntheses¹⁹.

Several reductive processes for the formation of 3-exomethylenecephams have recently been reported. These methods normally utilise simple precursors in a one-step reaction and are therefore particularly attractive on an industrial scale. 3-Chloromethyl cepheids (88) can be reduced⁶⁹ to the 3-exomethylenecepham (89) by electrolysis in either an aqueous THF/LiClO₄/NH₄ClO₄ or aqueous MeCN/EtOH/LiClO₄/NH₄ClO₄ system using a lead cathode. Yields of 80-90% are achieved and the reduction has also been applied to 3-iodomethyl and 3-benzothiazolyl cepheids.

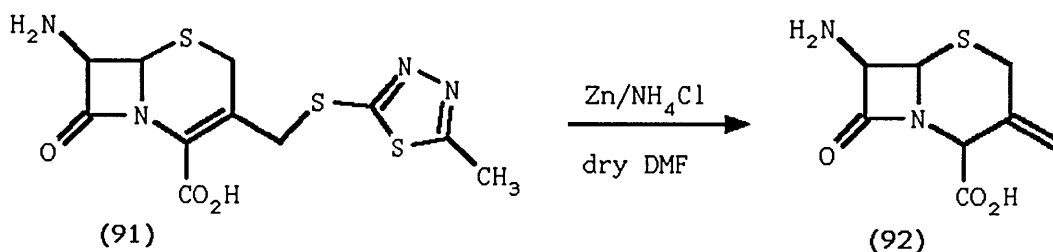


3-Halomethylcephams have also been chemically reduced⁷⁰ to the exomethylene derivative by treatment with CH₃CONHSi(CH₃)₃ which results in elimination of hydrogen halide. 3-(Heterocyclothio)methyl cepheids have also undergone elimination with this reagent to yield exomethylenecephams⁷⁰.

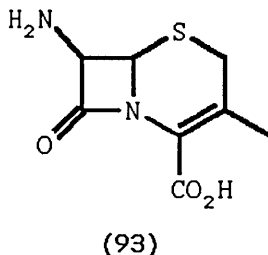
3-Acetoxymethylcephems (**90**) have previously been reduced electrochemically⁷¹ to the exomethylenecepham. Subsequently, chemical reduction with activated Zn/NH₄Cl has also been found⁷² to produce exomethylene derivatives (**86**) in 50-80% yield, depending on the C-7



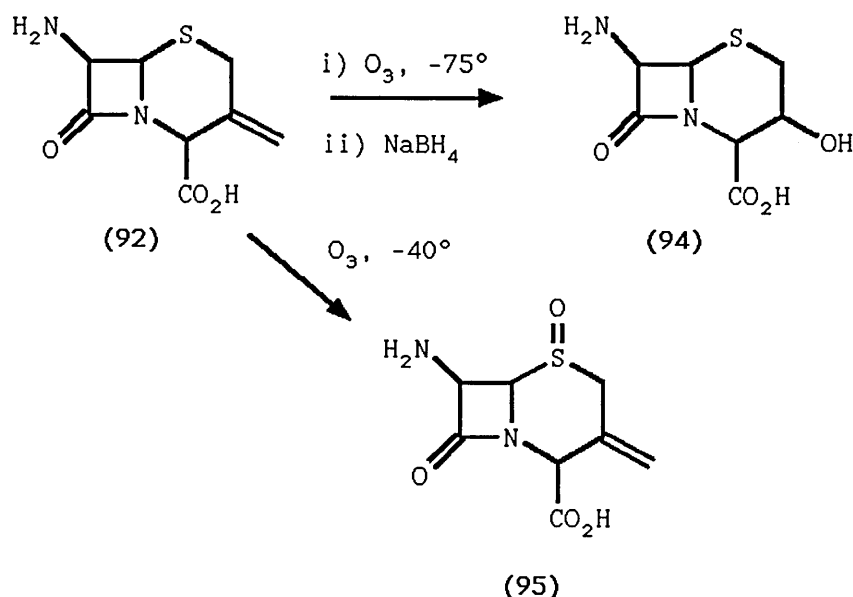
side-chain employed. In a detailed study⁷³ of the synthesis of 3-exomethylenecephams, without protecting groups at the C-4 carboxyl or C-7 amide positions, activated zinc/NH₄Cl has been found to be the reagent of choice for reduction of the 3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl derivative (**91**). The use of



acidic reducing conditions such as Zn/HCl or Zn/H₂SO₄ in either water or 50% aqueous DMSO, MeOH or AcOH resulted in formation of isomers (**92**) and (**93**). The yields of



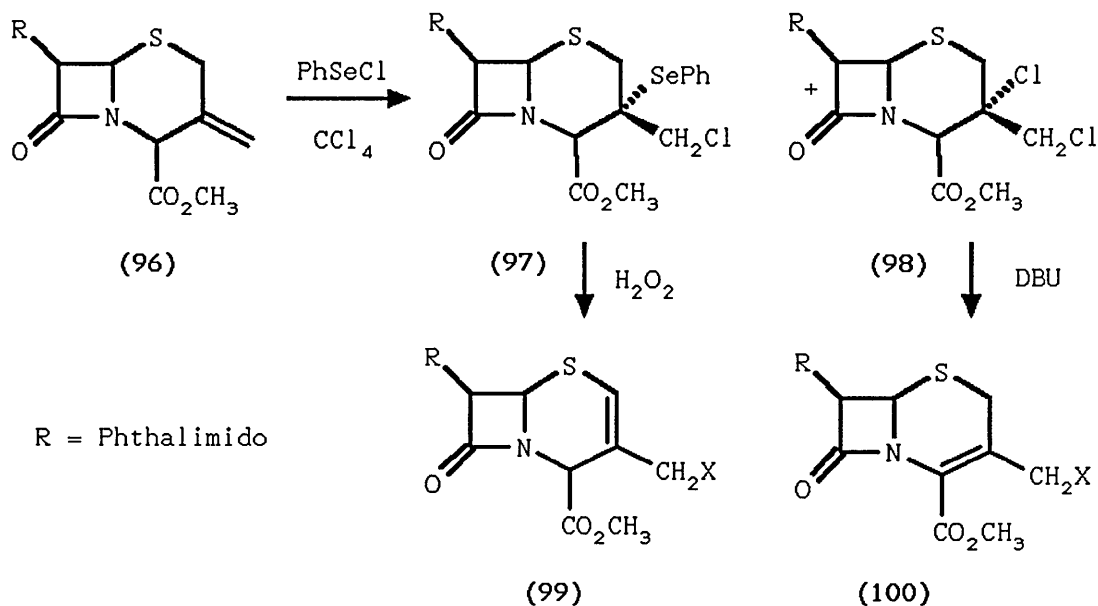
(92) and (93) varied between 44-78% and 42-12% respectively, depending on the conditions employed. Ozonolysis of the exomethylenecepham (92) as the methanesulphonate or hydrochloride, followed by reduction with NaBH_4 in basic aqueous solution, resulted in the 3-hydroxycepham (94) in high yield. Increasing the reaction temperature resulted in a competitive reaction between ozonolysis and oxidation at sulphur to produce sulfoxide (95). Formation of the sulfoxide prevents subsequent ozonolysis at C-3, and the authors postulate that this is due to steric hindrance by the sulfoxide moiety.



3-Exomethylenecephams are primarily used for conversion to the 3-hydroxy derivatives, however a number of interesting reactions of these exocyclic double bond systems have recently been reported. Addition of both phenylselenenyl chloride⁷⁴ and bromine⁷⁵ to the double bond system has been studied by Botta and

co-workers.

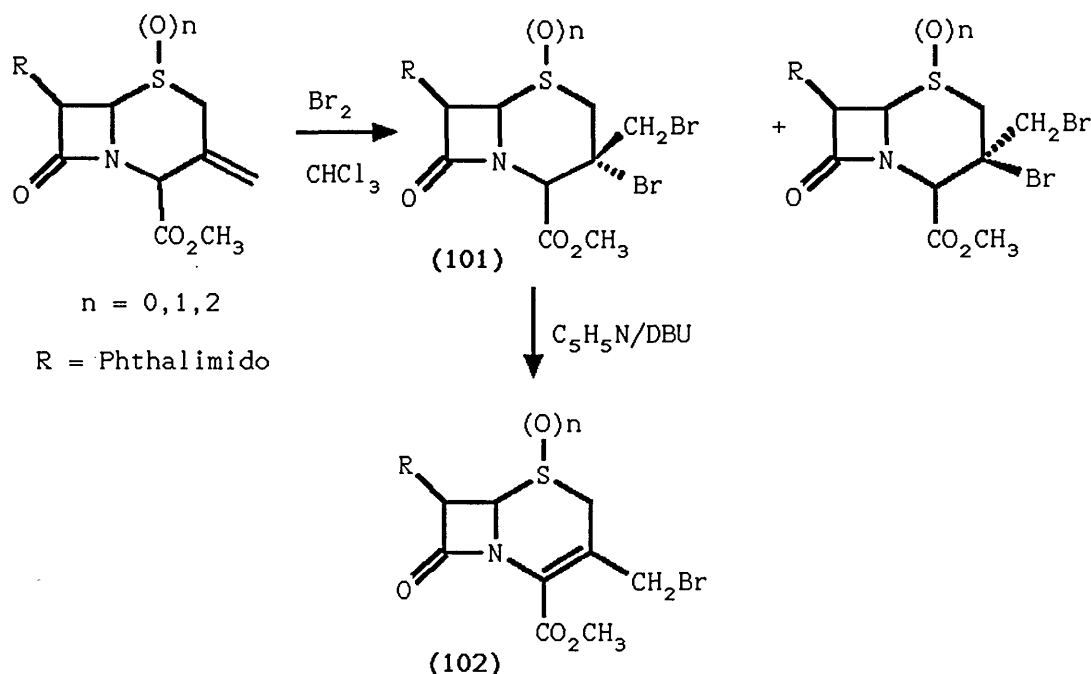
When 3-exomethylenecepham (96) is stirred with PhSeCl in CCl₄, the 3 α -seleno derivative (97) is the major product, and the dichloro adduct (98) is also obtained in low yield. Elimination of PhSeH from (97) *via* oxidation with H₂O₂ results in the 3-chloromethyl ceph-2-em (99; X=Cl), whereas dehydrohalogenation of (98) in the presence of DBU gives the 3-chloromethyl ceph-3-em (100; X=Cl). Both chloromethyl derivatives were trapped with methanol to give (99; X=OMe) and (100; X=OMe). When (96) was reacted with PhSeCl in refluxing



dichloroethane, (98) was obtained as the major product (81%). The authors suggest that at increased reaction temperatures, thermodynamic control of the addition reaction results in the dichloro derivative (98).

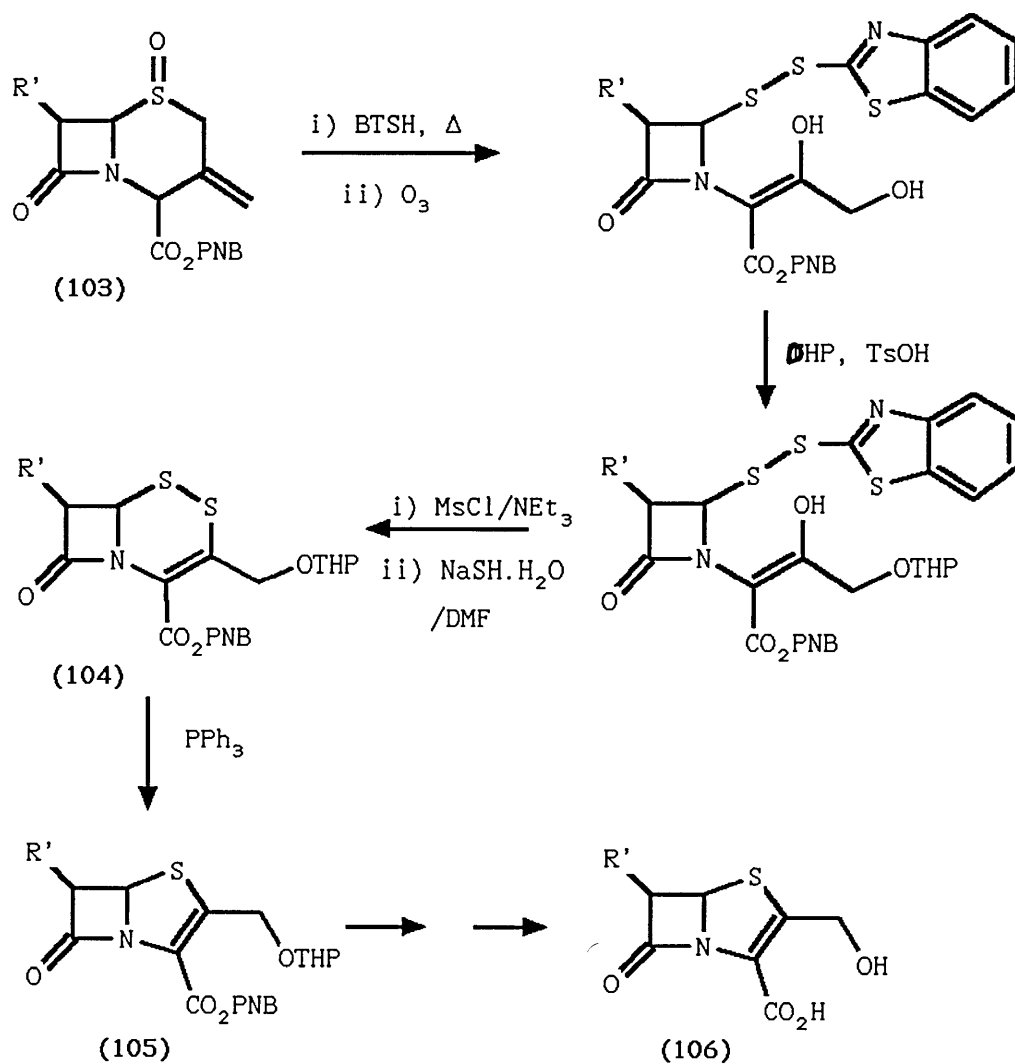
Addition of bromine to 3-exomethylenecepham sulphones, α -sulfoxides and sulphides has resulted in 3 β -bromo-methyl-3 α -bromocephams (101) as the major product. In

all cases, dehydrohalogenation with pyridine/DBU affords the 3-bromomethylceph-3-em (102).



The authors report that bromination occurs as expected for a double bond and that previously reported anomalies are a consequence of bulky C-4 ester groups⁷⁶.

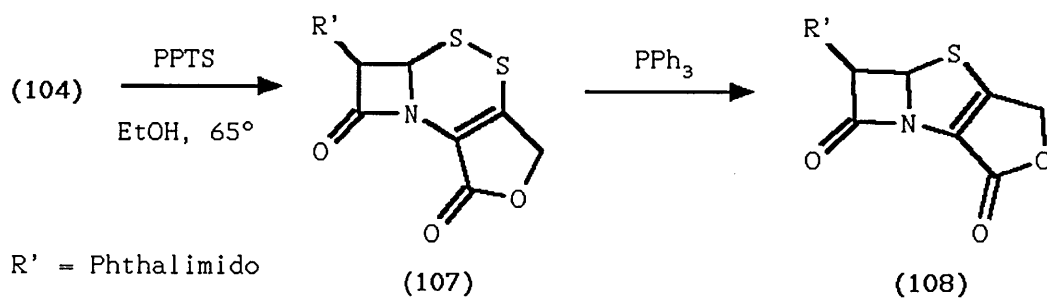
3-Exomethylenecephams have been used to synthesise 2-thiacephems⁷⁷ (which can subsequently be converted into penems⁷⁷⁻⁷⁹) and oxacephems^{80, 81}. Thus, 2-thiacephem (104) is formed by initial ring opening of the 3-exomethylenecepham (103) with 2-mercaptobenzo-thiazole (BTSH) under heating, followed by reaction as outlined in Scheme 2. Desulphurisation of the 2-thiacephem with triphenylphosphine affords the protected penem (105) which is deprotected using normal reagents to yield acid (106). Attempts to remove the



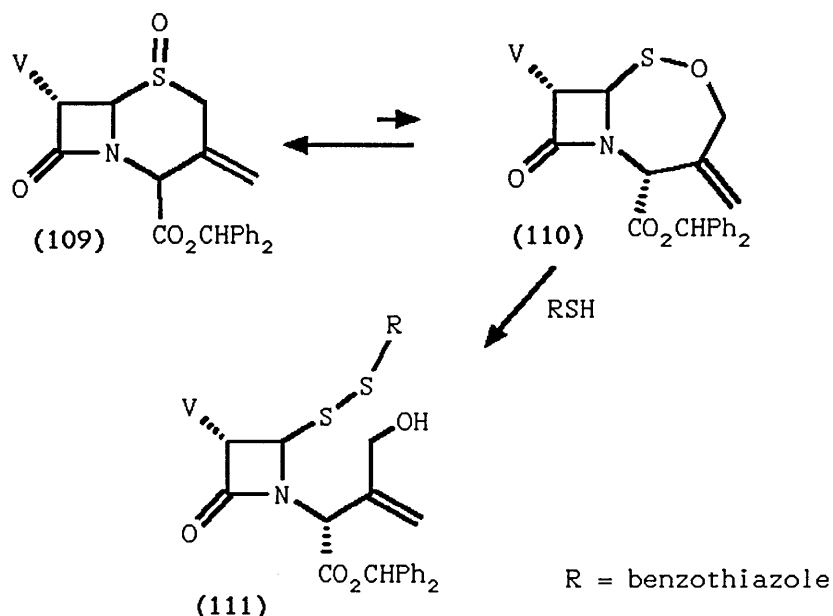
$\text{R}' = \text{Phthalimido}$

Scheme 2

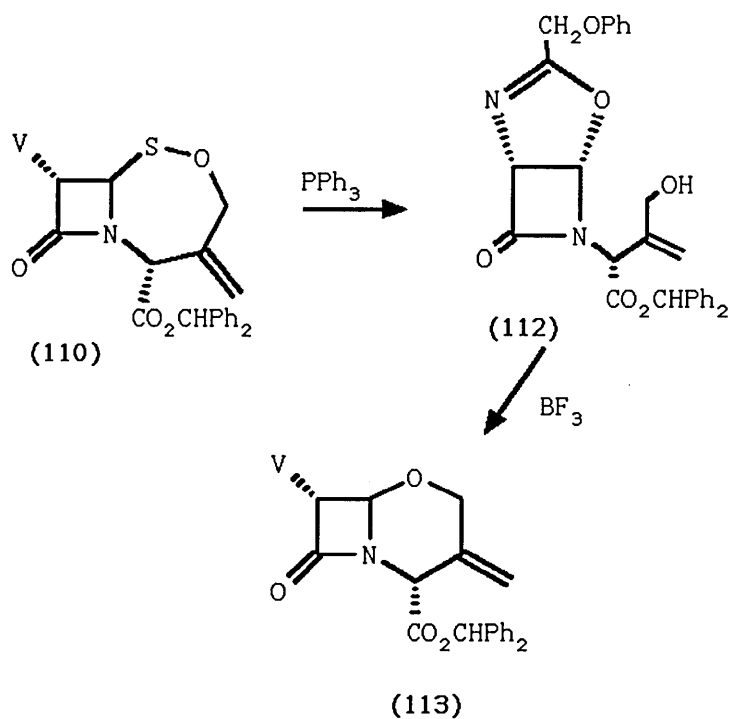
tetrahydropyran protecting group from (104) under mild conditions (PPTS , EtOH , 65°) resulted in sole formation of tricycle (107) which underwent ring contraction when reacted with PPh_3 to form tricyclic penem (108).



Cooper *et al*⁸⁰ have extended previous work⁸¹ which detailed the formation of azetidinone disulphide alcohols (111) from 3-exomethylenecephams *via* sulphenate (110). Subsequent reaction⁸⁰ of intermediate (110) with

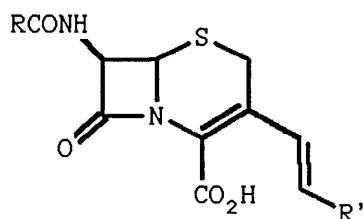


PPh_3 gave the oxazoline-azetidinone (112)⁸² which was converted into the corresponding 1-oxacephem (113) using BF_3 .

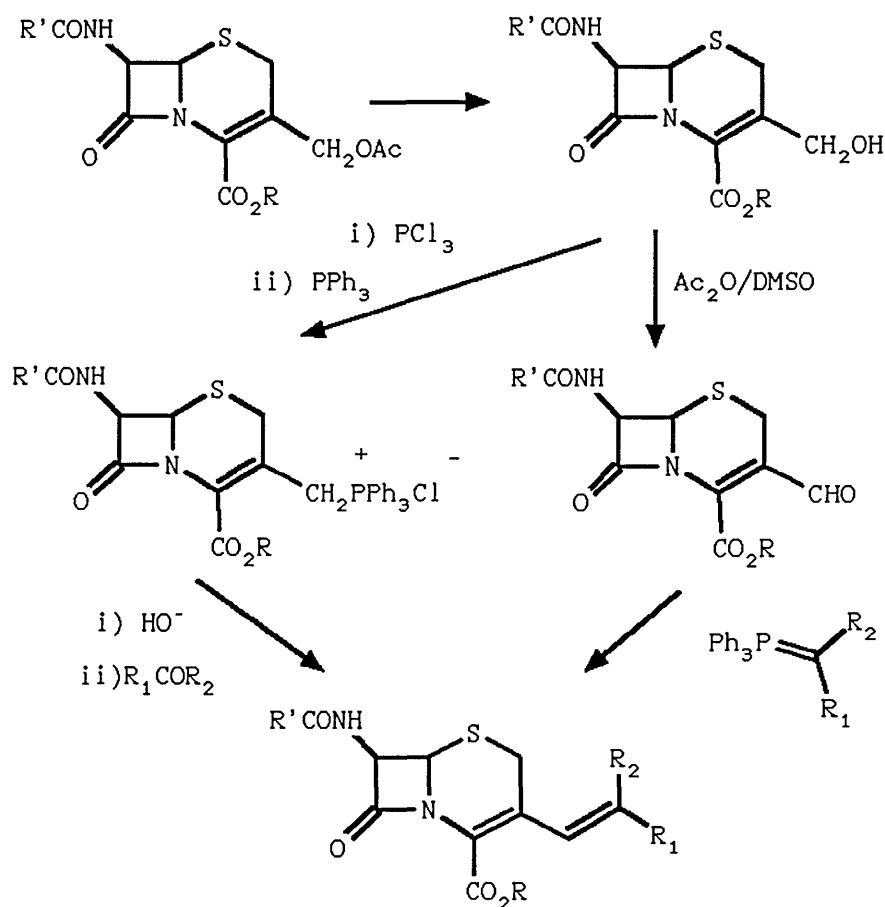


1.4.2) 3-Vinylcephalosporins

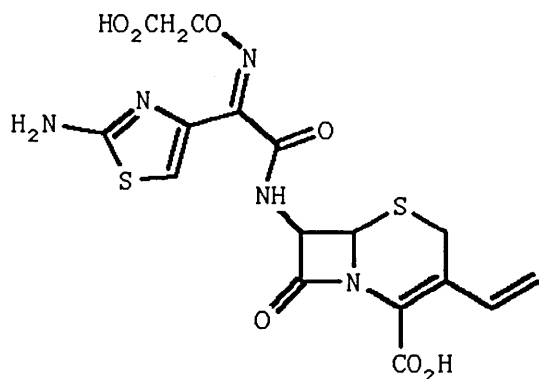
Cephalosporins bearing a 3-vinyl substituent have been widely studied following the discovery that compounds of type (114) have good antibacterial activity when R' is an electron-withdrawing group. Routes to 3-vinyl-



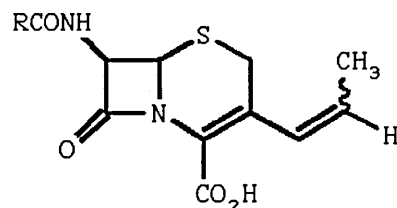
cephalosporins have previously been reviewed⁸³. The main route is *via* the 3-acetoxymethylcephalosporin as shown in Scheme 3.



A number of 3-vinylcephalosporins currently under extended biological evaluation have been prepared via this reaction scheme, for example, cefixime (115)⁸⁴ and BMY 28100 (116)⁸⁵. A wide variety of 3-vinyl-



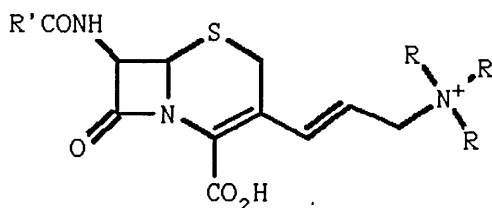
(115)



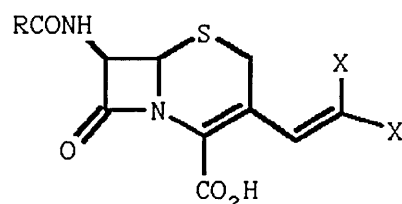
R = *p*-hydroxyphenylglycine

(116)

cephalosporins have shown good antibacterial activity. Thus, 3-(3-ammonium-1-propenyl) derivatives (117) have been found⁸⁶ to inhibit penicillin-resistant *staphylococci* and *pseudomonas* strains, whereas dihalovinyl cephems (118) have high activity against



(117)

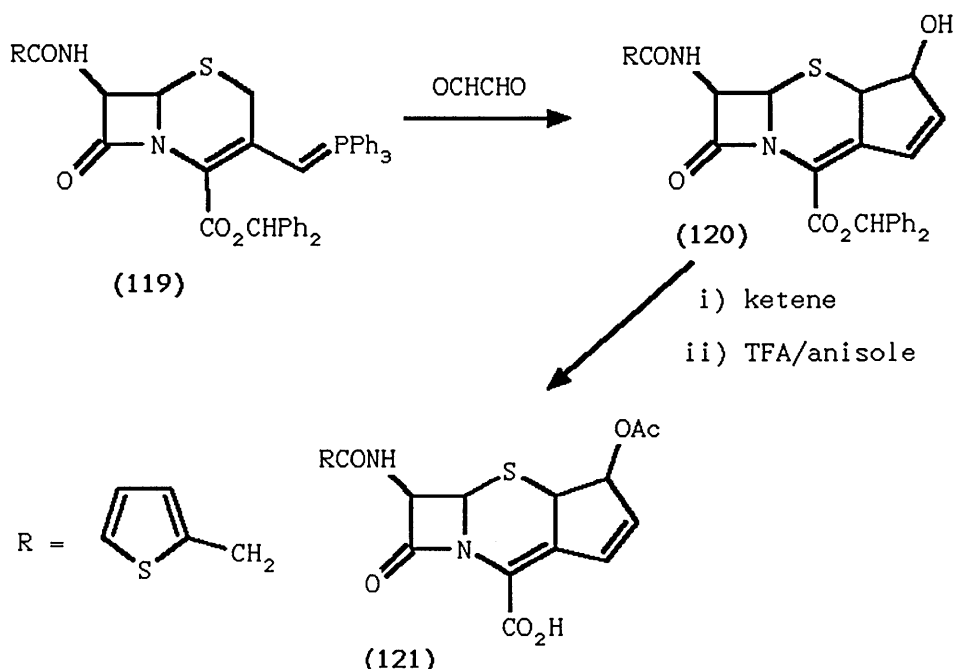


(118) X = Cl, Br, F

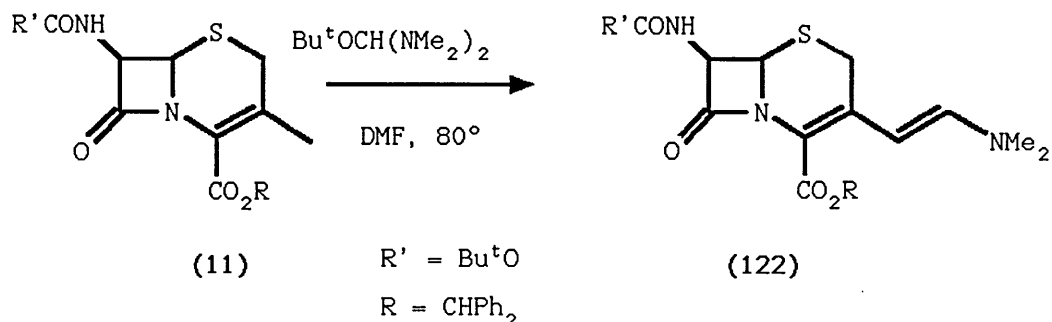
both Gram positive and Gram negative bacteria, but are only poorly enterally absorbed⁸⁷.

The phosphonium ylid (119), used as an intermediate in the synthesis of 3-vinylcephalosporins, has been found⁸⁸ to react with glyoxal to form tricyclic cephem derivatives (120) which could be acylated with ketene to form acetoxy derivatives (121) which showed some

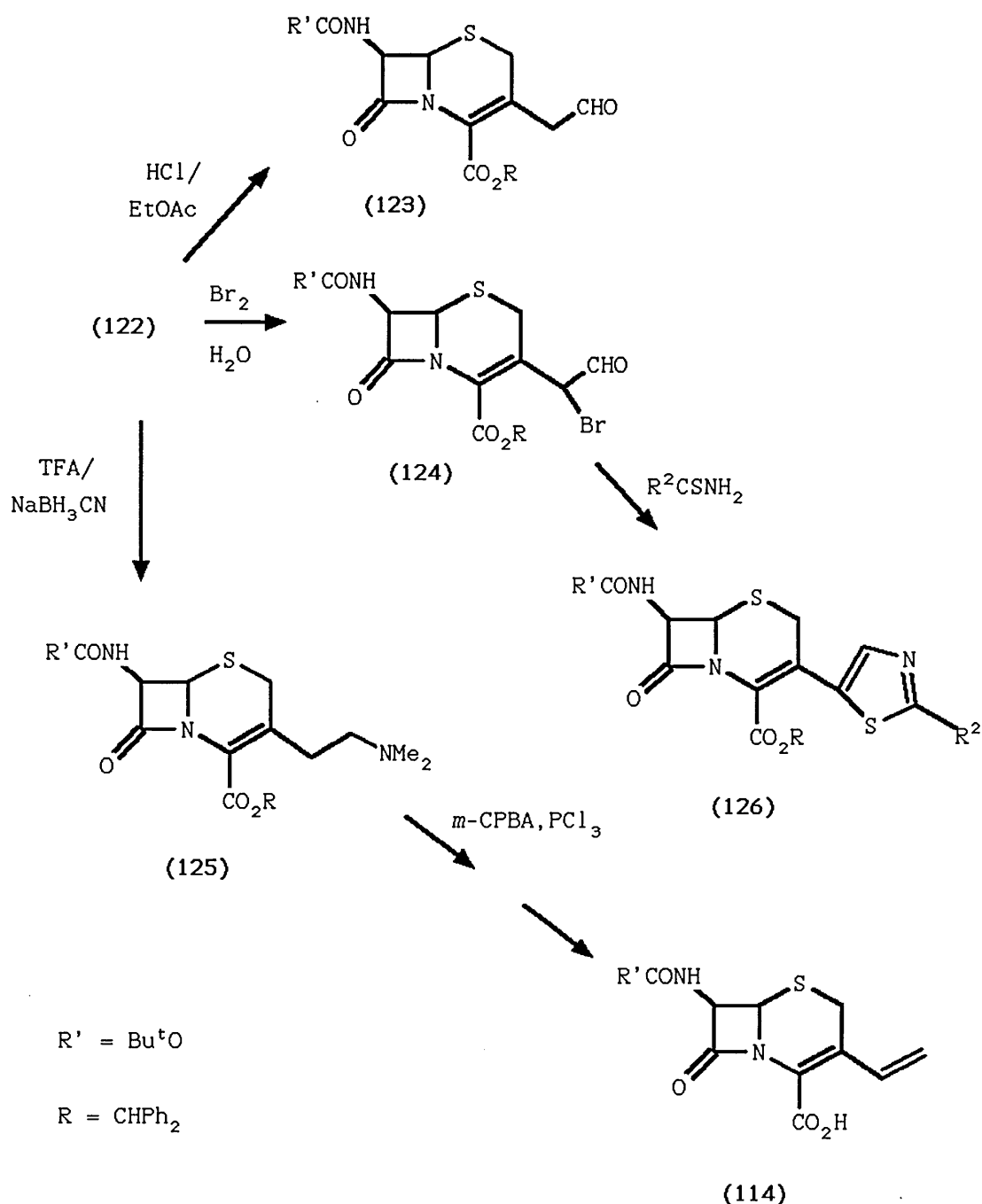
activity against Gram positive organisms.



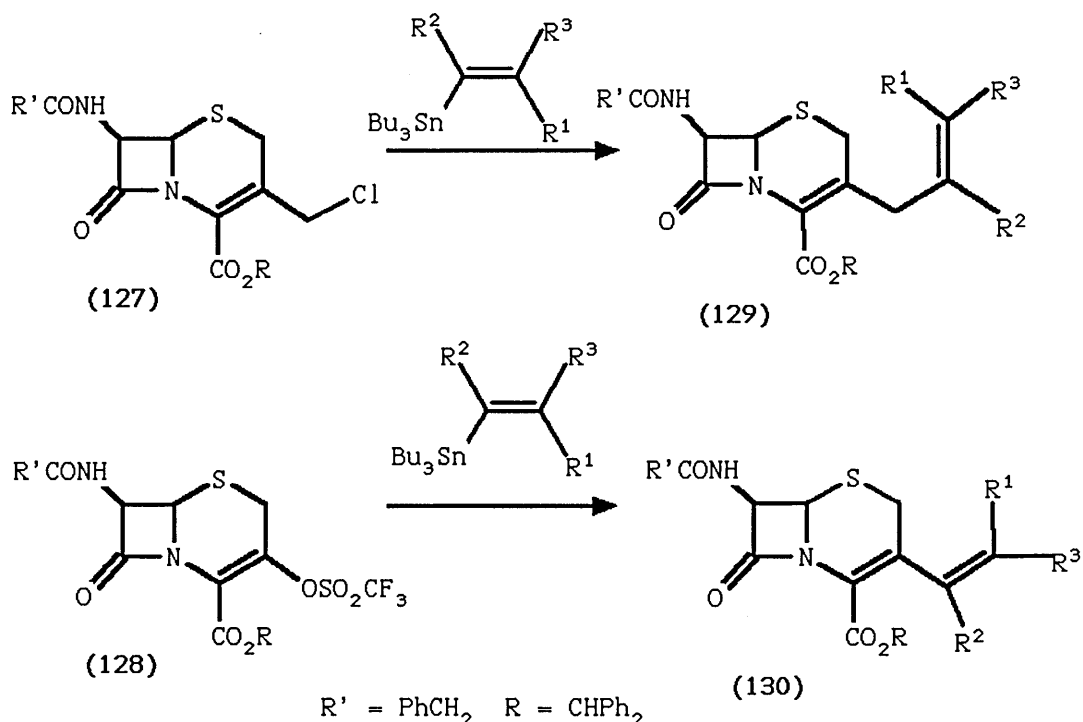
An alternative strategy for the formation of 3-vinyl-cephalosporins involves the reaction of C-3 methylcephems with ortho-amides to give the intermediate enamine (122)⁸⁹. This enamine undergoes a variety of



reactions to produce aldehyde (123), bromoaldehyde (124) and saturated amine (125). All of these undergo further reactions, for example, (124) reacts with thioamides to give heterocycles (126). The vinylcephalosporin (114) is synthesised from (125) via the unstable sulfoxide N-oxide, which undergoes a Cope elimination.

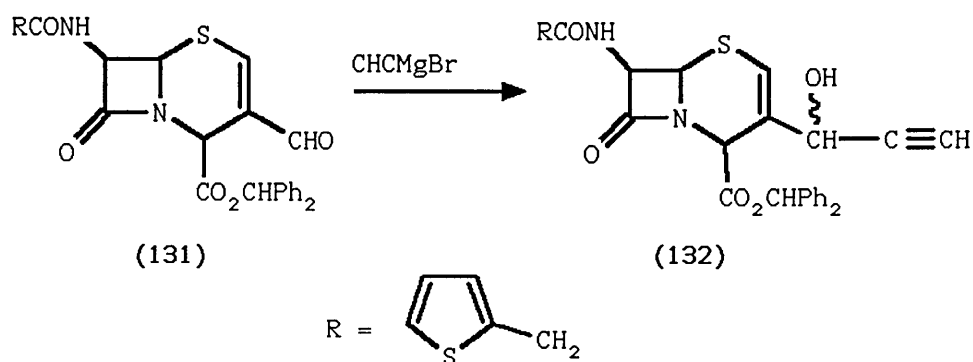


Very recently, the formation of cepheams with C-3' allyl substituents has been reported^{90, 91}. Unsaturated stannanes are coupled to either 3-chloromethylcephems (127) or the 3-triflate (128) in the presence of Pd catalysts to afford a wide range of derivatives with general structures (129) and (130) respectively. The

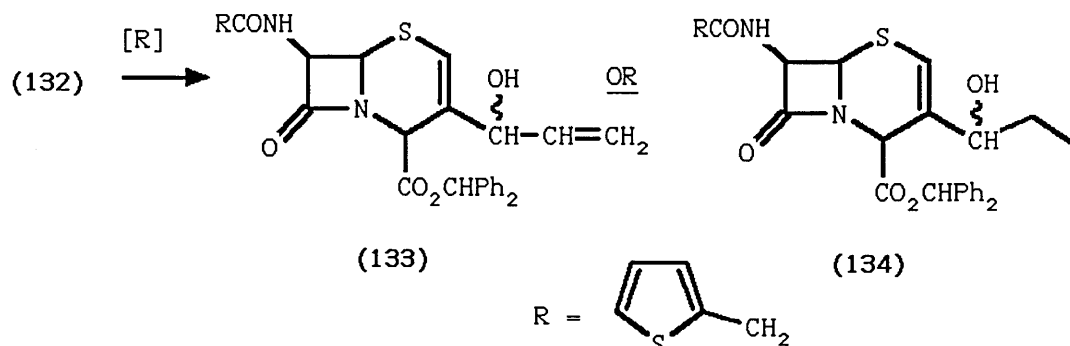


most effective catalyst was found to be produced when tri(2-furyl)phosphine was added to a solution of bis(dibenzylideneacetonyl)palladium in THF in the case of (127), or N-methylpyrrolidinone in the presence of ZnCl_2 in the case of (128). The authors report that the reaction is compatible with a wide variety of stannanes.

The unsaturated alcohol (132), prepared *via* reaction of the 3-formylceph-2-em (131) with an alkyne Grignard reagent, has proved to be a versatile intermediate for the incorporation of several C-3 substituents⁹².

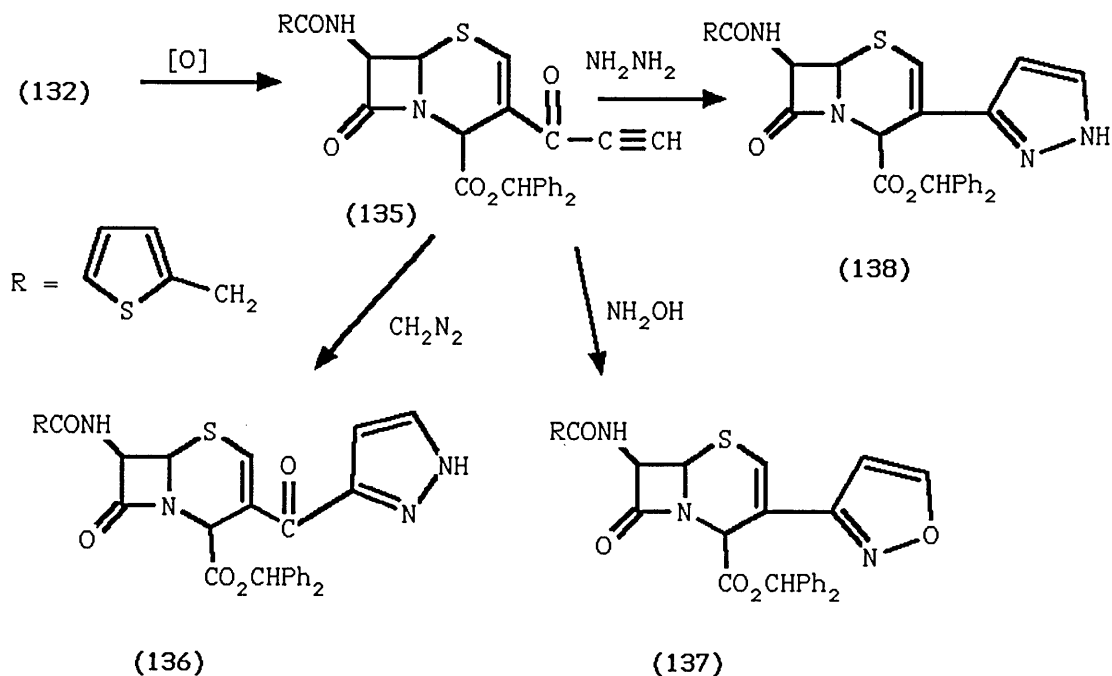


Reduction of (132) gave either the allyl alcohol (133) using Pd/BaSO₄/pyridine/H₂, or the saturated alcohol (134) with Pd/CaCO₃/H₂. Reaction of (134) with



PBr₃/pyridine followed by de-esterification, afforded the 3-vinylcephalosporin (114).

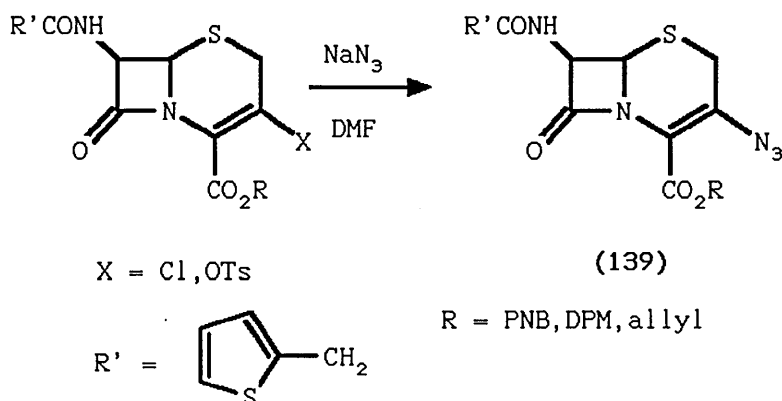
Oxidation of the alkyne alcohol (132) with chromic acid gave the ketone (135) in good yield. Further reaction with diazomethane afforded pyrazole (136). With hydroxylamine, (135) reacted to give isoxazole (137) and reaction with hydrazine yielded pyrazole (138), which was found to have some antibacterial activity.



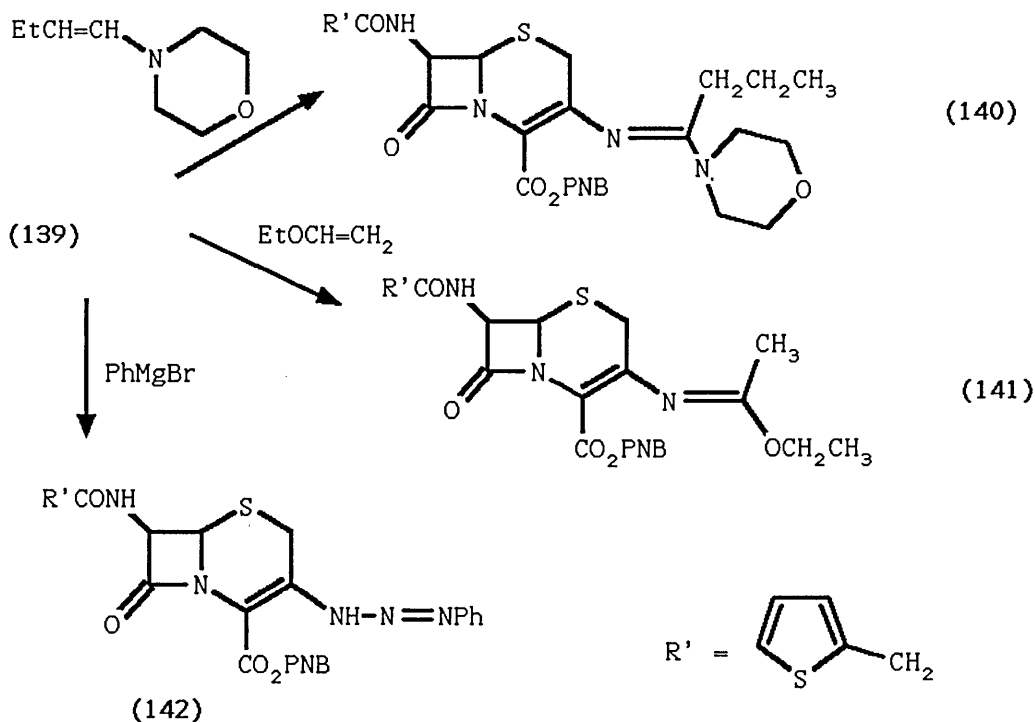
1.4.3) MISCELLANEOUS 3-SUBSTITUENTS

a) 3-Azidocephems

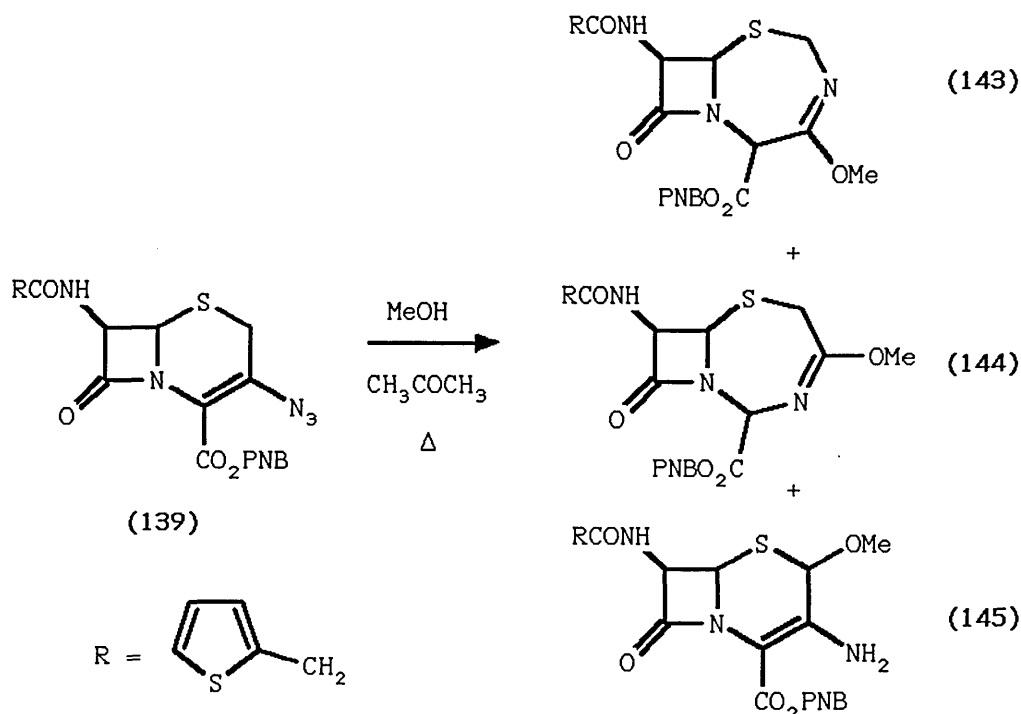
Azide displacement of either chloro- or tosylate substituents at the 3-position of cephems gives rise to 3-azidocephems (139)⁹³ which have electron deficient azide groups due to delocalisation *via* the Δ -3 double bond.



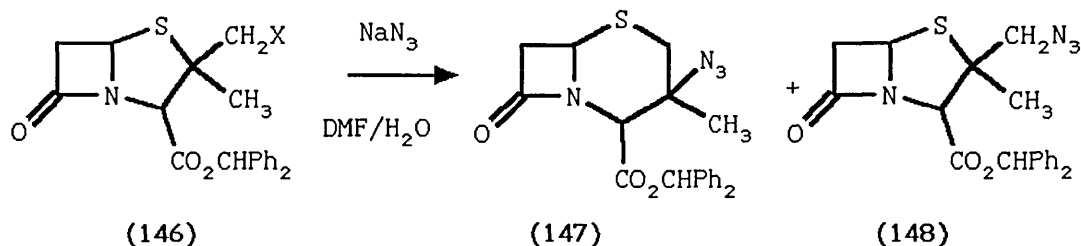
The azides react⁹⁴ to form amidines (140) and imidates (141) and also give triazines (142) when reacted with Grignard reagents. 3-Azidocephems have also been



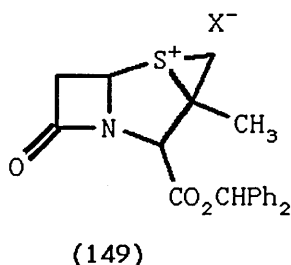
found⁹⁵ to undergo ring-expansion reactions when treated with methanol in refluxing acetone⁹⁵. Thus, cephem (139; R=PNB) gives esters (143), (144) and (145) under these



conditions. The de-esterified derivatives did not show any significant biological activity. In addition to the synthesis of 3-azidocephems from azide displacement reactions, 3-azidocephams (147) can be prepared⁹⁶ from 2-halomethylpenams (146) using sodium azide in aqueous DMF. The 2-azidomethylpenam (148) is also produced, the ratio of (147) to (148) being temperature dependent.

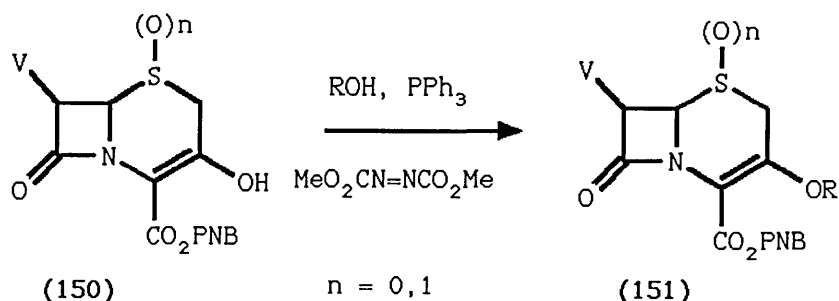


Attempts to displace the halide group of 2-halomethyl penam sulfoxides and sulphones with azide failed, leading the authors to believe that the reaction proceeds *via* thiirane intermediate (149) which could not be formed with oxidised precursors.



b) 3-Alkoxycephems and 3-Alkylcephems

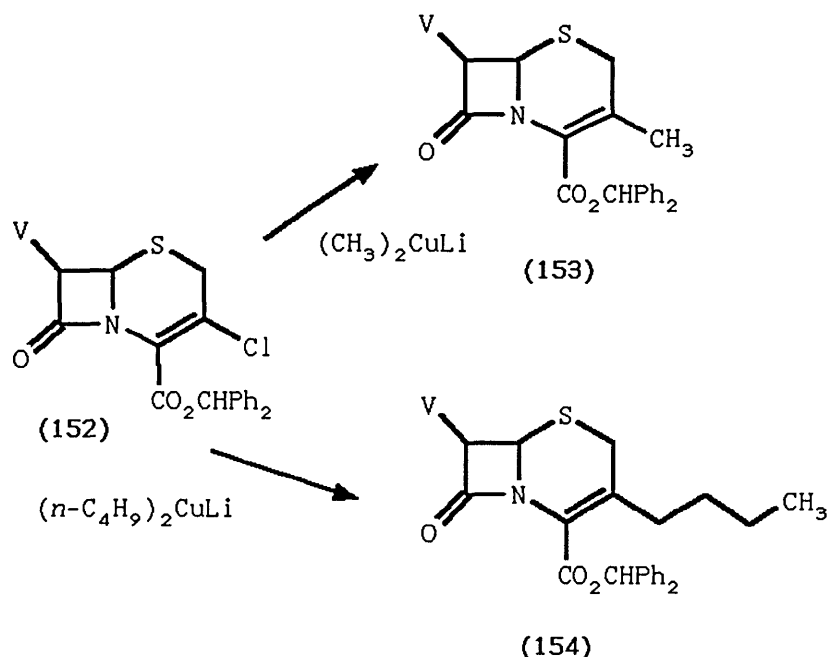
O-Alkylation of 3-hydroxycephems using a variety of reagents such as dimethyl sulphate, alkyl halides and Meerwein's reagent have been reported^{97,98}. A novel synthetic route to 3-alkoxycephems has also been described^{99,100} by Spry. 3-Hydroxycephem (150) undergoes a Mitsunobu reaction with alcohols in the presence of dimethyl azidodicarboxylate and triphenylphosphine to give the O-alkylated product (151) in good yield. A number of these O-alkylated cephems



have shown antibacterial activity, in some cases similar to that of cephalexin.

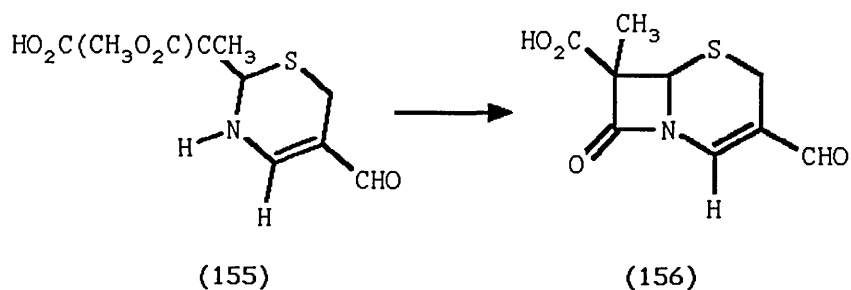
Alkylation of cephems bearing a variety of 3-substituents has been achieved using lithium cuprate

reagents¹⁰¹. Addition of lithium dimethylcuprate or lithium di-*n*-butylcuprate to 3-chlorocephem (152) gives the 3-methyl (153) and 3-*n*-butyl (154) derivatives respectively.



c) 3-Substituted cepems from thiazines

Several publications¹⁰²⁻¹⁰⁴ from a French group have detailed the conversion of substituted thiazines to cephem derivatives. Thus, 3-formylcephem (156) was prepared from thiazine (155) in the presence of 1-benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate.



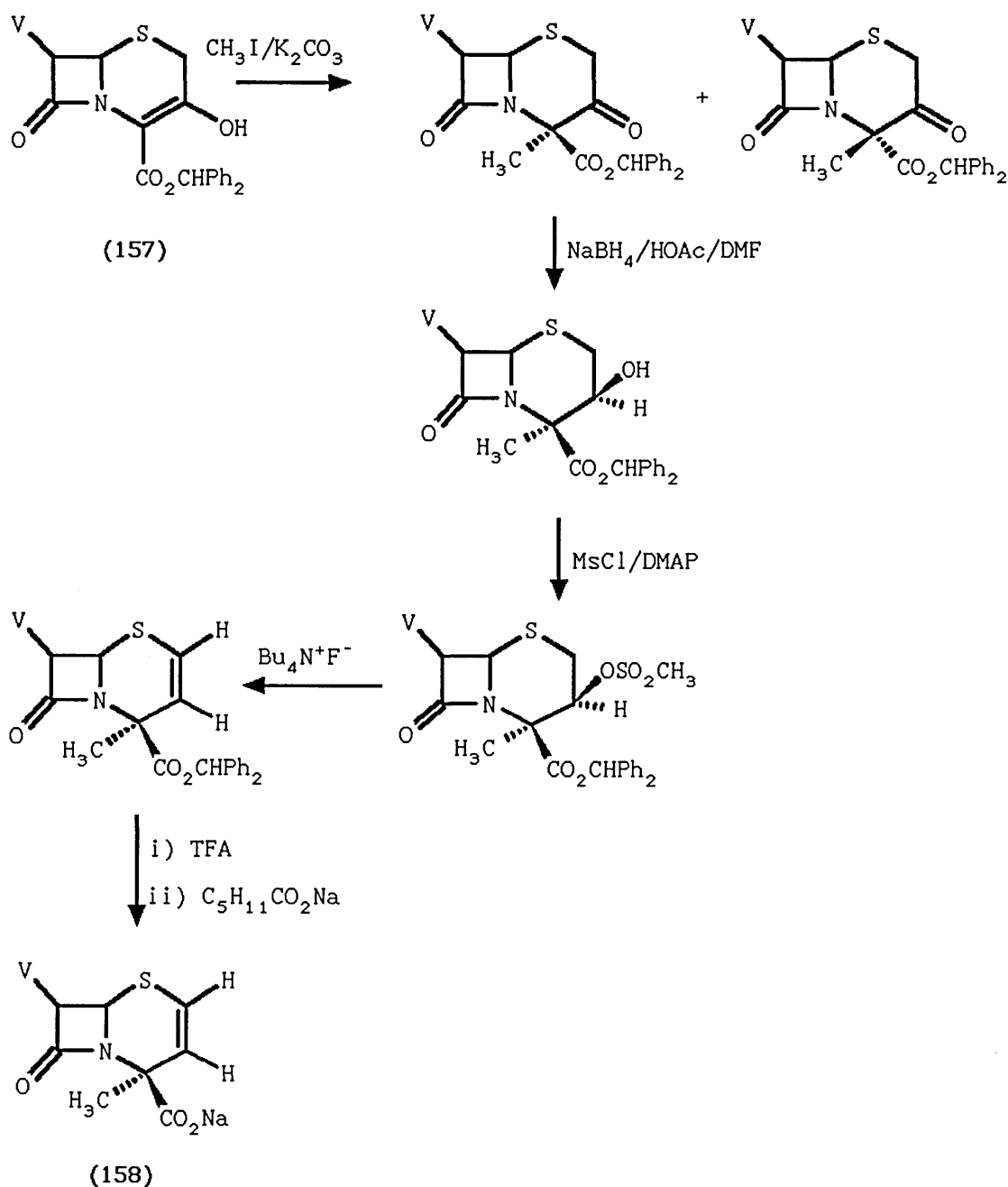
1.5) REACTIONS AT C-4

Modifications at the 4-position in cephalosporins have been carried out by two main routes. Firstly, direct chemical manipulation of the existing C-4 carboxylic acid gives rise to a variety of C-4 substituted cephalosporins¹⁰⁶. Alternatively, addition of groups to the 4-position *via* base-induced deprotonation at C-2 followed by delocalisation of the negative charge to C-4 gives rise to C-4 disubstituted ceph-2-ems⁴⁶.

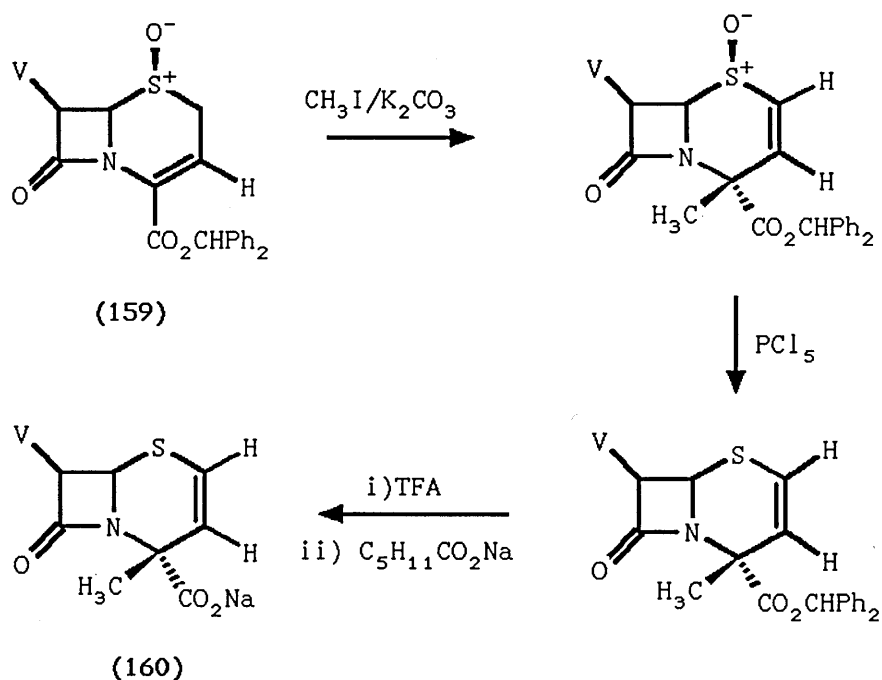
Ceph-2-ems are biologically inactive, however, and Cohen¹⁰⁶ has investigated these systems in an attempt to rationalise this lack of activity. The initial hypothesis that the β -lactam moiety in ceph-2-ems is in some way less reactive than in the corresponding ceph-3-em isomer had previously been discounted by Frere *et al*¹⁰⁷ who had shown that the likelihood of hydrolysis of the Δ -2 and Δ -3 cephems was similar. After analysis of 3-D positional parameters for several active and inactive β -lactams, Cohen concluded that the lack of activity in Δ -2 cephems was primarily due to a misfit with the highly specific 3-D recognition sites on the transpeptidases involved in cell wall biosynthesis. This misfit was thought to be a consequence of the α -orientation of the C-4 carboxyl group and it was therefore proposed that ceph-2-ems with a C-4 β -oriented carboxylic acid functionality would show antibacterial

activity.

Subsequent research from the same group¹⁰⁸ has attempted to confirm this hypothesis. Formation of the 4 β -carboxylic acid salt (158) was effected *via* a multi-step synthesis from the 3-hydroxycephem (157), utilising a 4 α -methyl substituent to ensure the carboxylic acid retained β -stereochemistry. Attempts to



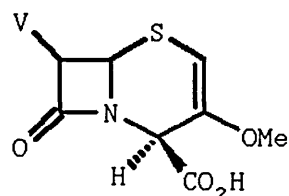
produce the corresponding 4 α -carboxyl-4 β -methylceph-2-em (160) *via* similar methodology failed due to problems encountered in eliminating the 3 α -mesylate group. (160) was, however, formed *via* a variation on existing C-4 alkylation techniques¹⁰⁹ from ceph-3-em sulphoxide (159).



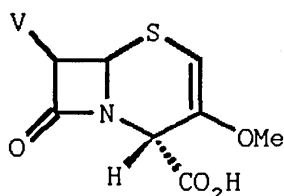
Both compounds (158) and (160) were found to have significantly reduced activity compared to the corresponding ceph-3-em acid when tested against a variety of organisms, with (158) proving to be entirely inactive against all organisms tested.

Further studies have been performed¹⁰⁸ on compounds (161) and (162) which were isolated as by-products in the synthesis of 3-methoxyceph-3-ems, *via* NEt₃ induced isomerisation. Although these compounds should almost exactly match the structural requirements proposed by Cohen, they were found to have higher minimum inhibitory

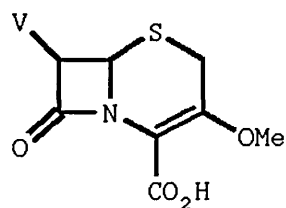
concentrations against a range of organisms than the 3-methoxyceph-3-em acid (**163**). The authors suggest that



(161)



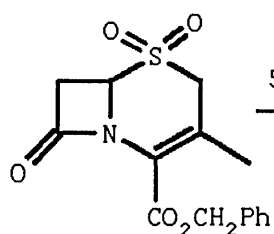
(162)



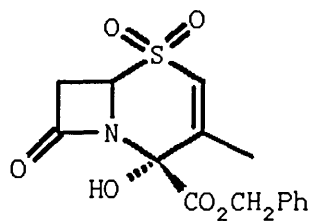
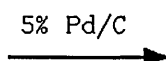
(163)

the lack of activity observed with ceph-2-ems is due to a combination of misfit between the cephem and the highly stereoselective transpeptidases and also as a result of diminished reactivity of the β -lactam system compared to the ceph-3-em series.

Several reports of novel ceph-2-ems have recently appeared. Stoodley and co-workers¹¹⁰ have observed an oxidative allylic rearrangement of the ceph-3-em sulphone (**164**) with 5% Pd/C in the absence of hydrogen to yield the 4-hydroxycephem (**165**). The structure of

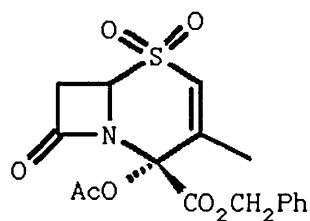


(164)



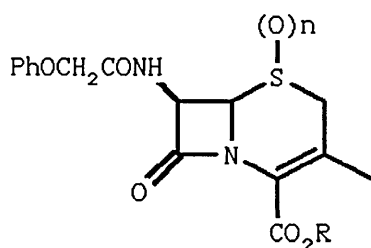
(165)

(**165**) was determined by spectroscopic methods, and *via* reaction with acetic anhydride/pyridine to afford the O-acetyl derivative (**166**). Both sulphone and ester



(166)

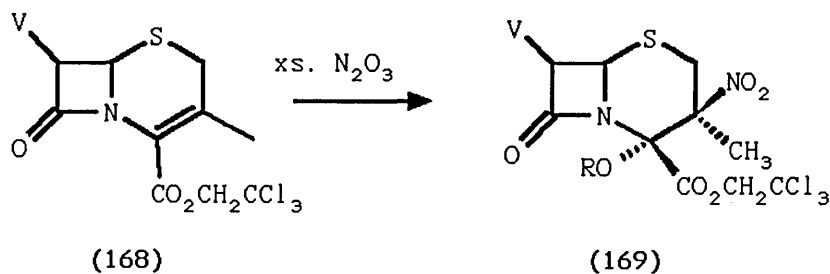
functionalities were found to be necessary for this rearrangement, as illustrated by the lack of reaction of cephems (167; $n=0,1$; $R=CHPh_2, H$) under the same conditions. Sulphone (167; $n=2$; $R=CHPh_2$) did react to



(167)

produce the corresponding 4-hydroxy derivative. In all cases, the 4-hydroxy group was found, by X-ray analysis, to be α -oriented. None of the sodium salts (prepared by ester hydrolysis followed by reaction with sodium 2-ethylhexanoate) showed any antibacterial or β -lactamase activity.

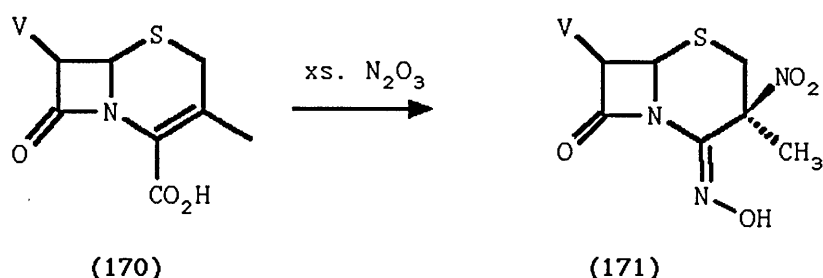
Hydroxylation of the cephem nucleus at C-4 has also been observed by Campbell *et al*¹¹¹ in the reaction of ceph-3-em carboxylates with dinitrogen ~~tetra~~^{tri}oxide. Ester (168) forms the 4-hydroxy-3-nitrocepham (169; $R=H$)



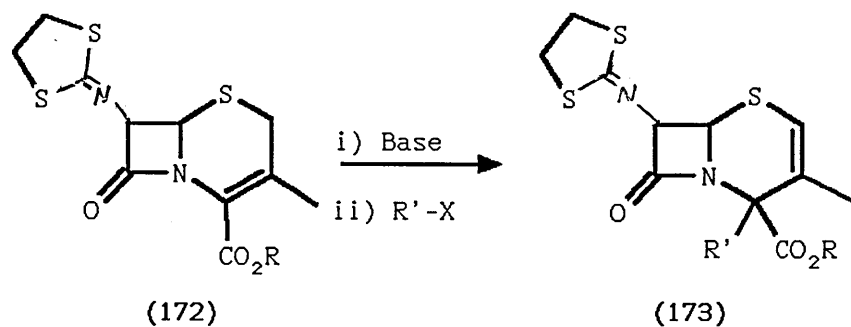
(168)

(169)

which being of limited stability was converted to the corresponding acetate (**169**; R=Ac) to facilitate analysis. The stereochemistry at the C-3 and C-4 positions was determined by nuclear Overhauser enhancement. Previous work by the same authors¹¹² has shown that an analogous reaction with ceph-3-em carboxylic acids proceeds *via* an alternative pathway. When acid (**170**) was stirred with excess N₂O₃ in dichloromethane, the 3β-nitro-4-oxime (**171**) was obtained.



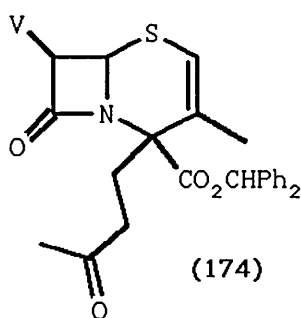
Routes to C-4 disubstituted ceph-2-em's have been reported *via* deprotonation at C-2, followed by trapping of the resultant delocalised negative charge at C-4. Thus, protected cephem (**172**) reacts¹¹³ with a variety of bases including NaH, LDA and KOBu^t to form anions which, on addition of a wide range of electrophiles, produce cephems of general structure (**173**). Depending on the



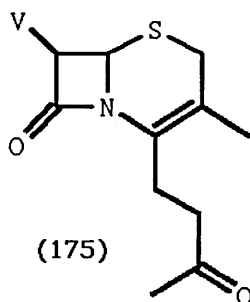
R' = alkyl, alkoxy, halomethyl,
(substituted)benzyl

reaction conditions and ester-protecting group employed, mixtures of *cis*- and *trans*- β -lactams were obtained in varying proportions. Cephems of type (173; R=H) were reported to be active against Gram positive and Gram negative bacteria.

In an extension to previous work¹¹⁴, Bremner and Ringan have examined⁴⁵ the reaction of ceph-3-ems with methyl vinyl ketone in the presence of triethylamine to afford ceph-2-em (174) in high yield. This paper also



reports a 3-step process for the removal of the C-4 carboxylate group, resulting in novel C-4 mono-substituted ceph-3-em (175). Further details of this



methodology are discussed in Section 2.1.4.

1.6) REACTIONS AT C-7

1.6.1) 7 α -Methoxycephalosporins

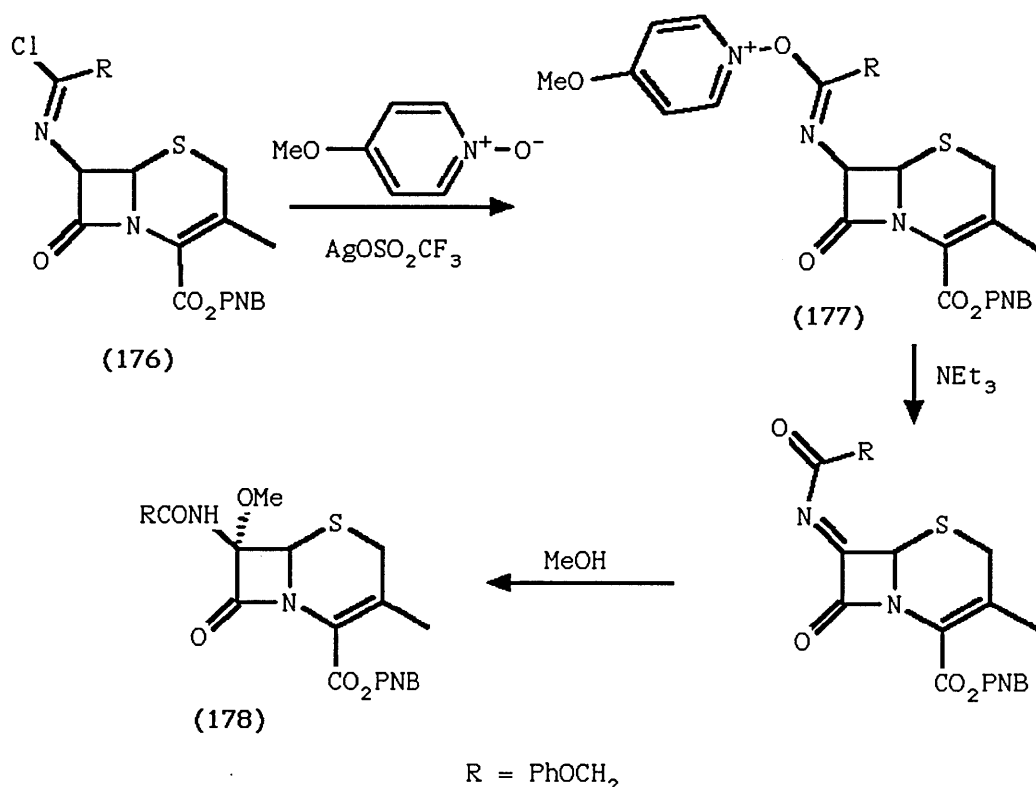
In general, strategies for obtaining novel active

β -lactams can be divided into two types:

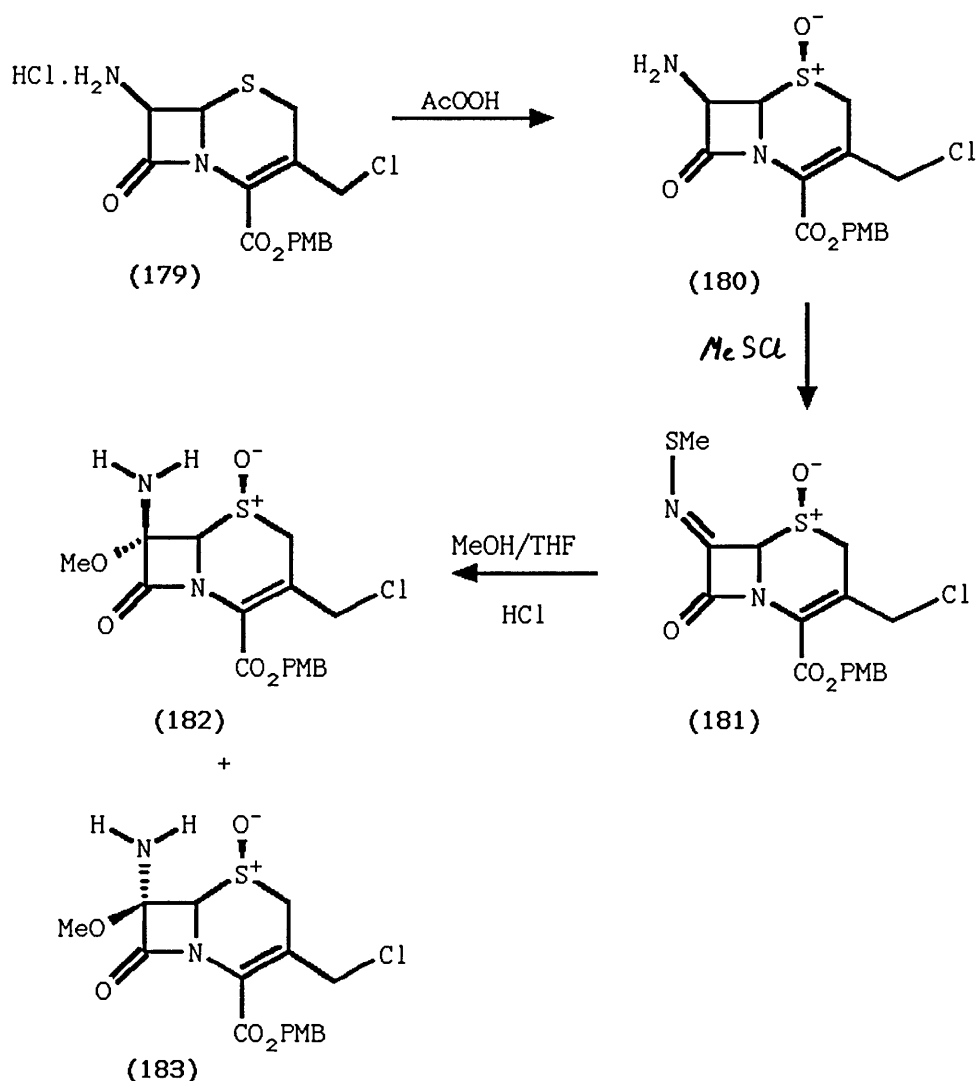
- screening of isolates from naturally occurring species;
- chemical modification of a known substrate.

The discovery of cephalosporins with a 7 α -methoxy substituent highlights the way in which these two strategies can work in tandem. The isolation of cephamycins (5) from *streptomyces* species by Lilly⁸ and Merck¹¹⁵ in 1971 was followed by the introduction of the methoxy group into cephalosporins by chemical synthesis¹¹⁶ in the following year. Several cephamycins, all having a 7 α -methoxy substituent and displaying enhanced β -lactamase stability, have subsequently been isolated^{117, 118} from natural sources.

A number of methods for the introduction of a 7 α -methoxy group have been reported and these have recently been reviewed¹¹⁹. Of particular interest is a 'one-pot' process¹²⁰ and an industrially viable method¹²¹ for the introduction of the methoxy group. The chloroimine (176) reacts¹²⁰ with 4-methoxypyridine N-oxide in the presence of silver triflate to give (177) which undergoes a 1,4-elimination with triethylamine, followed by addition of methanol to the intermediate acylimine to yield (178). The majority of processes for



the 7 α -methoxylation of cephalosporins are either low-yielding or require reagents which preclude their use on an industrial scale. To overcome this problem, Shionogi workers¹²¹ have developed a simple stereospecific 7 α -methoxylation process. Oxidation of the amine hydrochloride (179) (prepared by deacylation of the corresponding 7 β -phenylacetamido compound) with peracetic acid affords the β -sulphoxide (180) which is converted to the sulphenimine (181) using methanesulphenyl chloride¹²². Treatment of (181) with methanol in THF containing 2 equivalents of HCl at -20°C affords the 7 α -methoxy amine (182) in 62% yield and the epimer (183) in 1% yield. The predominant formation of

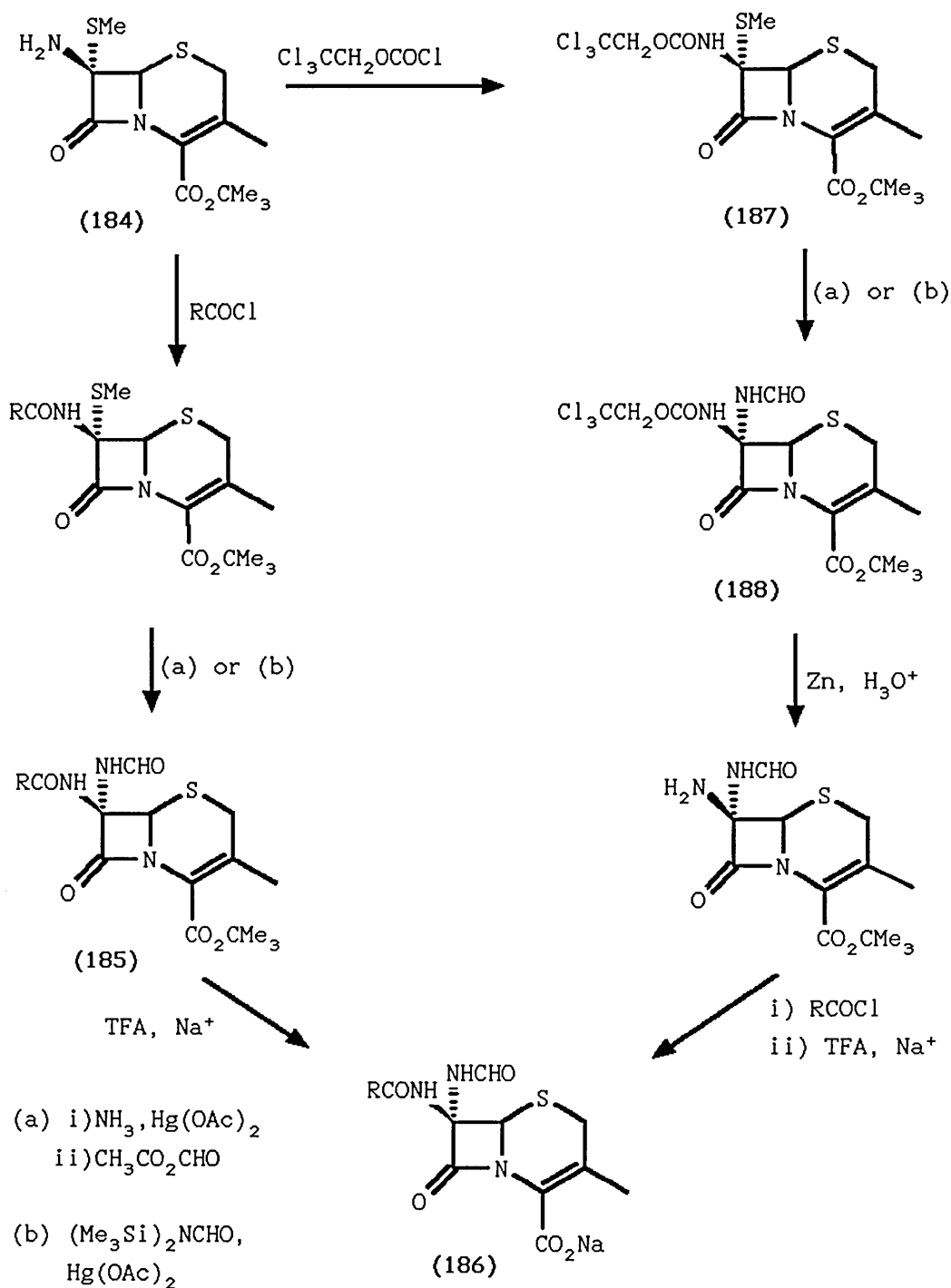


the 7 α -methoxycephem can be rationalised by the presence of a hydrogen bond between the 7 β -amino group and 1 β -sulphoxide oxygen in **(182)**, thus hindering nucleophilic attack on the β -face, and also stabilising the product formed from nucleophilic attack on the α -face.

1.6.2) 7 α -Formamidocephalosporins

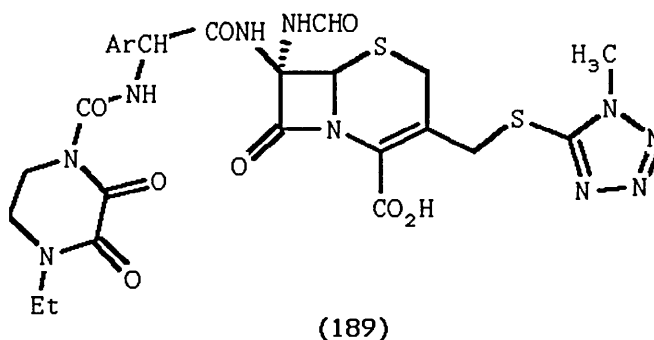
The interest in 7 α -substituted cephalosporins, arising from the isolation of the cephamycins, prompted a wide study of alternative 7 α -substituents, of which only a

few examples have exhibited any antibacterial activity (see Section 1.6.3). The major exception to this lack of activity has been the 7 α -formamido series first reported by Beechams in 1984¹²³, and subsequently found to be produced naturally by a variety of organisms¹²⁴. The 7 α -formamido group is introduced using the 7 α -thiomethylcephem (**184**) as a precursor. Two alternative routes have been devised, differing only in the methodology employed to introduce the 7 β -amido group. Thus, direct acylation of (**184**) followed by reaction with either NH₃/Hg(OAc)₂ and CH₃CO₂CHO¹²⁵, or N,N-bis(trimethylsilyl)formamide/Hg(OAc)₂¹²⁶ results in 7 α -formamidocephem (**185**) which is converted into salt (**186**). Alternatively, protection of the 7 β -amine as the trichloroethoxycarbonyl derivative (**187**) followed by formylation as before affords 7 α -formamidocephem (**188**). Deprotection of the amine followed by acylation and de-esterification affords salt (**186**). A wide range of



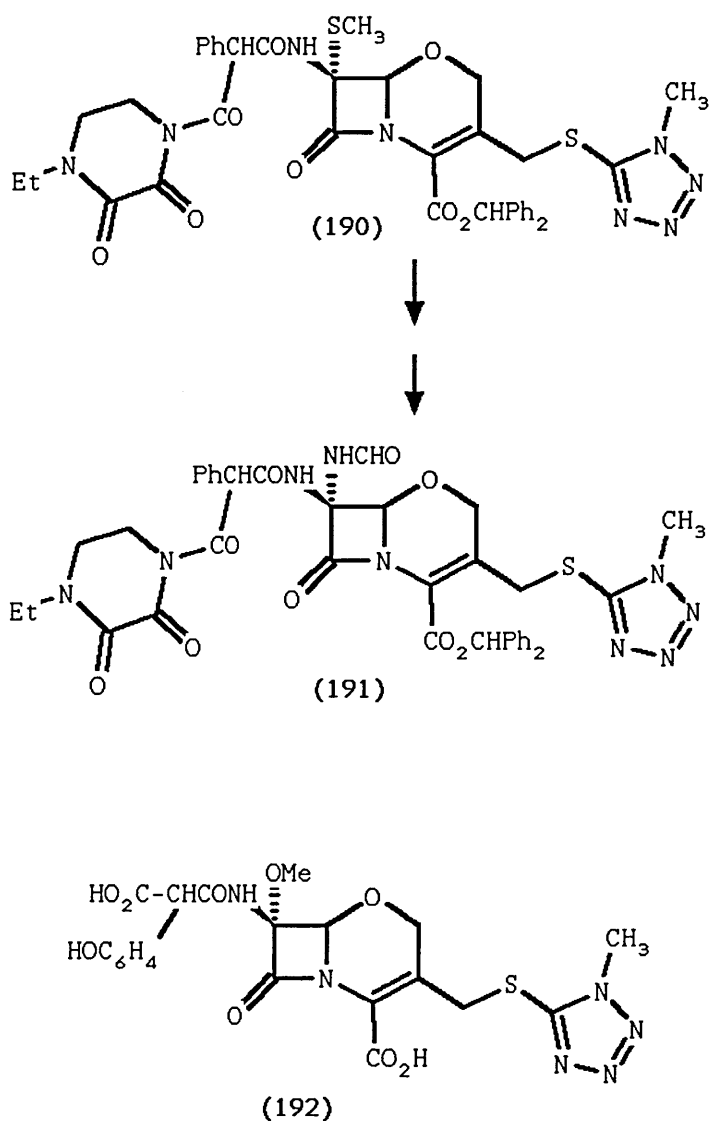
7-amide side-chains and C-3' substituents including (heterocyclothio)methyl and pyridinium groups have been incorporated into the 7 α -formamido nucleus. Of the 7-amido groups studied, much research^{29, 127, 128} has concentrated on derivatives of type (189). These

compounds retain¹²⁷ the stability toward β -lactamases observed in the 7 α -methoxycephalosporin series, but with enhanced antibacterial activity toward a broad spectrum of organisms. In addition to the N-7 and C-3'



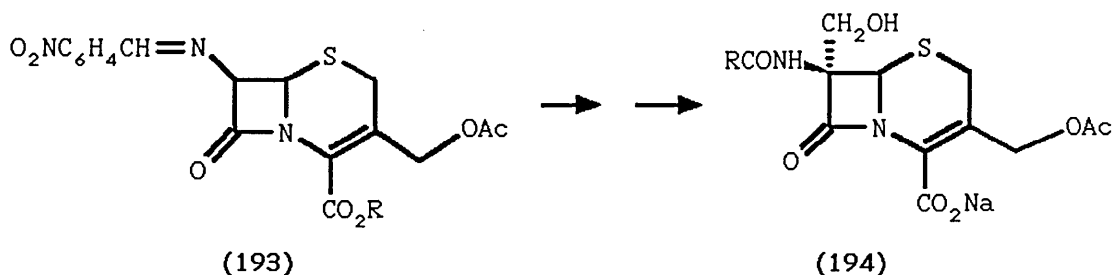
substituents, the oxidation state and resultant stereochemistry of the sulphoxide has been shown²⁹ to affect the activity of the molecule.

Extension of the methodology used to introduce 7 α -formamido groups into cephalosporins has been applied to the oxacephalosporin series. Oxacephalosporin (190) was converted into the 7 α -formamido analogue (191) which was found to have similar activity to the corresponding cephalosporins, and was more active than the 7 α -methoxy-oxacephalosporin latamoxef (192).



1.6.3) Other 7 α -substituents

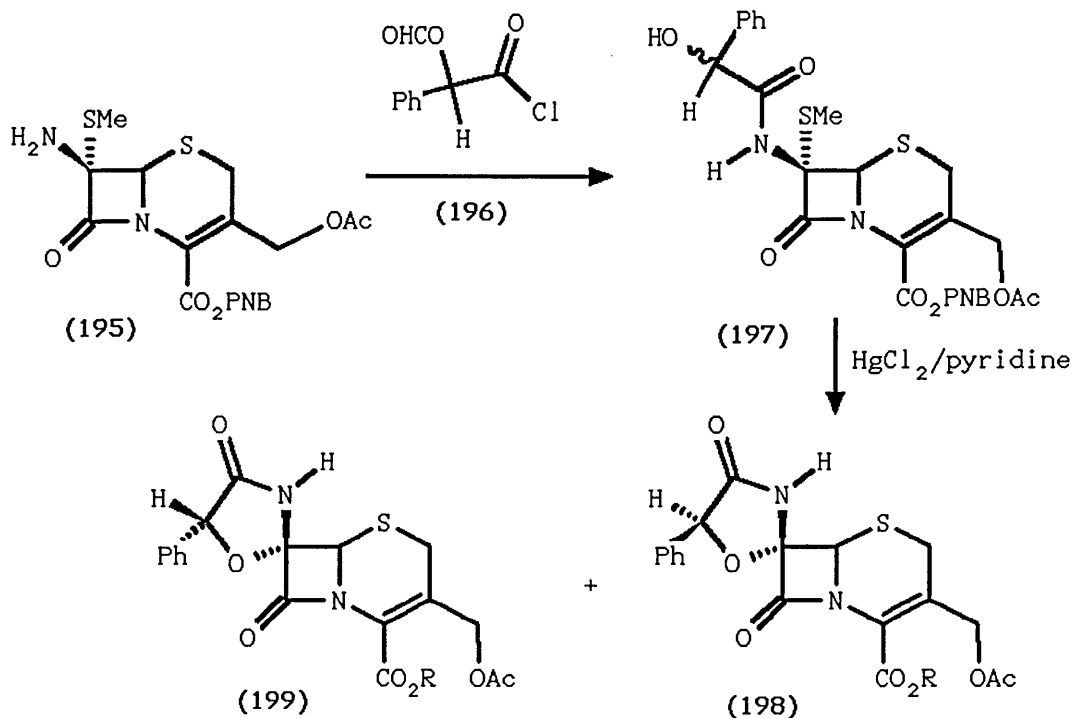
Other than 7 α -methoxy and formamido substituents, only 7 α -hydroxymethyl substituents have been found¹²⁹ to possess any antibacterial activity. Thus, treatment of the 4-nitrobenzylidene Schiff's base (193) with K₂CO₃, followed by quenching with HCHO at -20°C, removal of the ester group, and acylation leads to the 7 α -hydroxymethyl derivative (194).



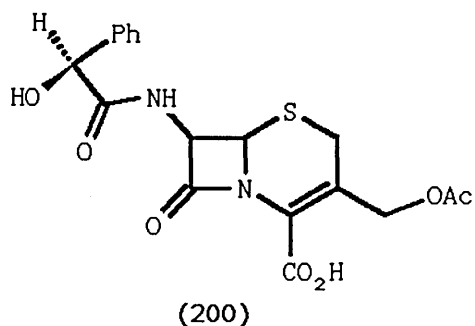
1.6.4) Reactions at N-7

The vast majority of reactions involving the C-7 amine group in 7-ACA derivatives are acylation reactions with suitable carboxylic acid derivatives to produce 7-amide side-chains.

However, the introduction of spirocyclic groups at C-7 has resulted^{130, 131} in compounds which show some anti-bacterial activity. Thus, reaction of 7 α -thiomethyl-7 β -aminocephem (195) with acid chloride (196) results¹³⁰ in alcohol (197) after removal of the formyl protecting group. Cyclisation with HgCl_2 and pyridine gives the spiro compounds (198; R=PNB) and (199; R=PNB) which are de-esterified to the corresponding acids (198; R=H) and (199; R=H). Only (199; R=H) showed any antibacterial



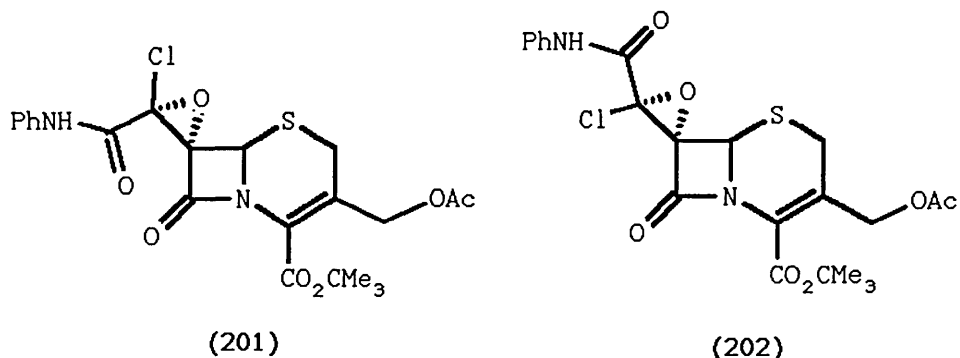
activity, but this is considerably decreased compared to the acyclic analogue (200). It is interesting to note



that the side-chain chiral carbons in (199) and (200) have differing stereochemistries, with the cyclic (S)-isomer being active whereas the acyclic (R)-isomer is active.

Spirocyclic side-chains have also been introduced¹³¹ as a result of an unusual reaction between 7-diazocephalosporins and oxalyl chloride. The structure of the 7-spiroepoxycephalosporin (201) was assigned on the

basis of ^1H & ^{13}C nmr and X-ray crystallography. Also isolated as a minor product was isomer (202) which was



found, after de-esterification, to have some Gram positive activity, whereas the (S)-isomer (201) was completely inactive when de-esterified. Similar reactions have been observed¹³² in the penicillin series¹³², where all of the spiroepoxide analogues of (201) and (202) were found to have β -lactamase inhibitory activity.

1.7) MISCELLANEOUS CEPHALOSPORIN DERIVATIVES

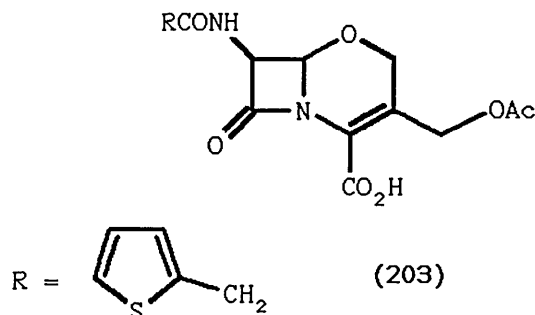
1.7.1) Nuclear Analogues

Although semi-synthetic penicillins and cephalosporins have been under development for some 30 years the overwhelming majority of all compounds studied have been prepared by modification of the C-6 (C-7) amide side-chain, or the C-3 methylene group in cephalosporins. Modifications to the basic bicyclic nucleus, either by the addition of substituents or by total synthesis, constitutes an area of wide possibility for structural modifications and the subsequent sections of this introduction will outline significant examples of these

'non-classical' cephalosporin analogues.

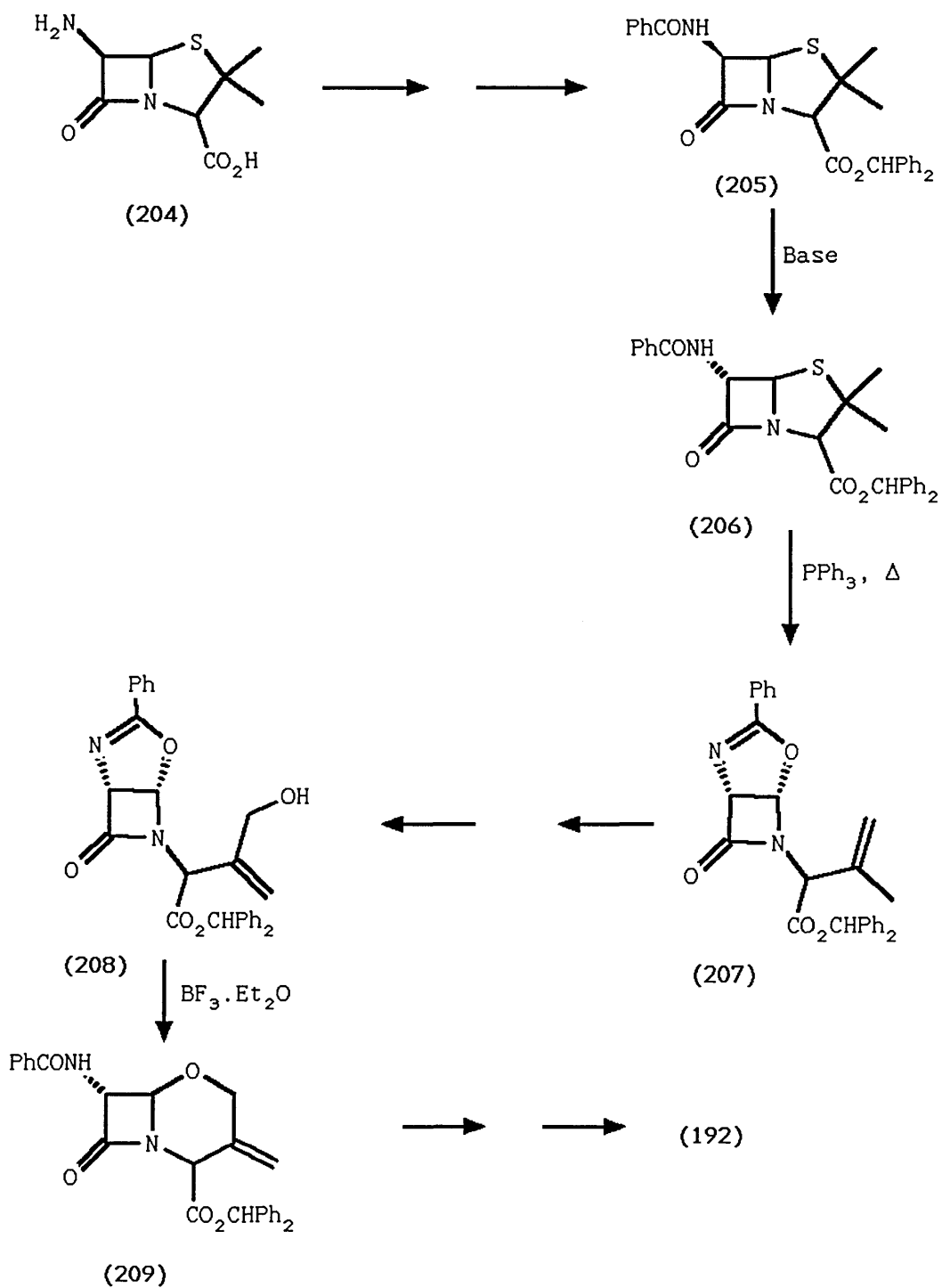
1.7.1.1) 1-Oxacephems

The preparation of a cephalosporin where the dihydrothiazine ring sulphur is replaced by an oxygen atom was first achieved by the total synthesis of 1-oxacephalothin (**203**) in 1974⁶. This compound had a



similar spectrum of activity to cephalothin, however its level of activity was more than two times greater, thus showing that the dihydrothiazine ring is not necessary for antibacterial activity. 1-Oxacephems have since been thoroughly investigated¹³³.

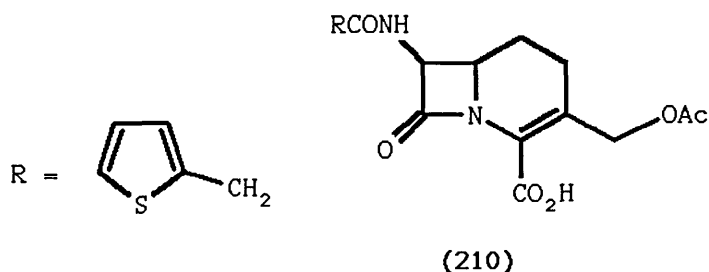
Synthesis of the dihydro-oxazine ring from monocyclic azetidinone precursors has been reviewed elsewhere¹³⁴. On an industrial scale, 6-APA (**204**) is often used as the starting material. Thus, in the synthesis of latamoxef (**192**)¹³³, the first broad spectrum 1-oxacephem, the acylated penicillin (**205**) is epimerised by base to give the *trans*- β -lactam (**206**) which is ring opened with triphenylphosphine to give oxazoline (**207**). Further manipulation of (**207**) results in (**208**) which is ring closed with BF_3 to give the oxacephem skeleton (**209**) which is then converted into latamoxef (**192**) by standard methods. The majority of 1-oxacephems which exhibit



biological activity contain a 7 α -methoxy group though recent work¹³⁵ has shown that this functionality is not always necessary.

1.7.1.2) 1-Carbacephems

In general, replacement of the sulphur atom in cephalosporins with a methylene group results in diminished levels of biological activity. Thus, the carbon analogue of cephalothin (210) has only half the

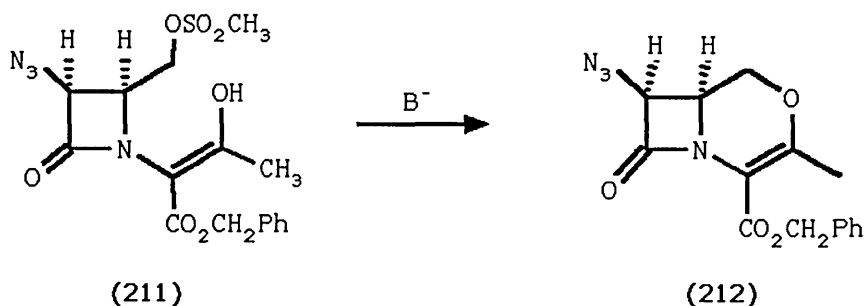


biological activity of the sulphur-containing molecule¹³⁶. Synthesis of the carbacephem bicyclic system has resulted in many innovative routes¹³⁷, and recently an enantioselective synthesis of the carbacephem nucleus has been reported¹³⁸.

1.7.1.3) Hetero-1-carbacephems

1-Carbacephems with a further heteroatom in the 6-membered ring have been synthesised by several groups and some derivatives display antibacterial activity.

2-Oxa-1-carbacephems (212) can be prepared by cyclisation of (211) with a base catalyst¹³⁹. The

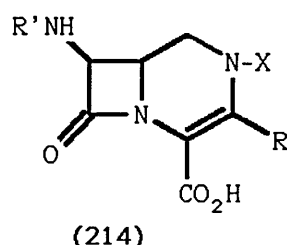
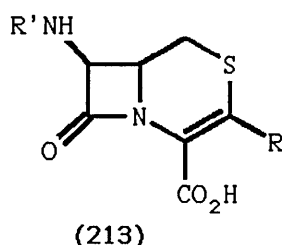


biological activity of acids derived from (212) can be enhanced by increasing the hydrophilic nature of the

3-substituent or, as in the case of cephalosporins, the use of 3(heterocyclothio)methyl groups.

2-Oxa-1-carbacephems (212) and 2-thia-1-carbacephems (213) have also been prepared from L-aspartic acid¹⁴⁰.

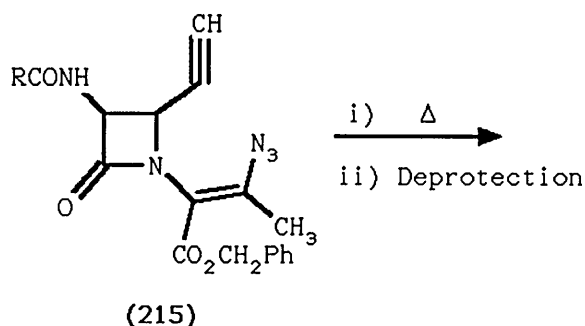
2-Aza-1-carbacephems (214) have also been synthesised¹⁴¹ and have been found to be more active than 2-thia-1-carbacephems. A recent review has surveyed the



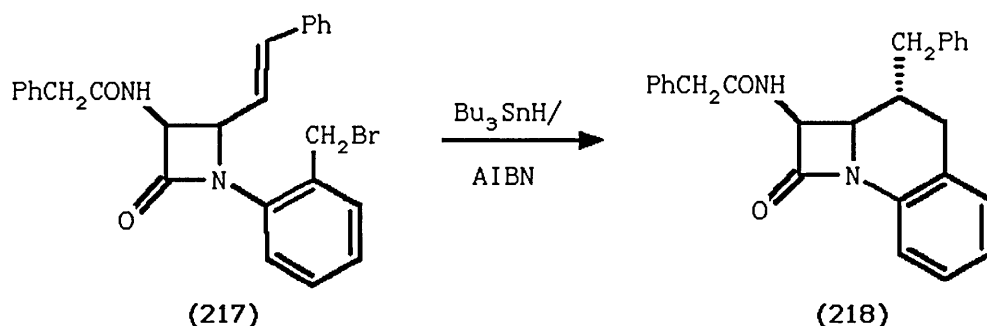
synthesis and activity of a wide variety of cephalosporin nuclear analogues¹¹⁹.

1.7.2) Tricyclic Cephalosporins

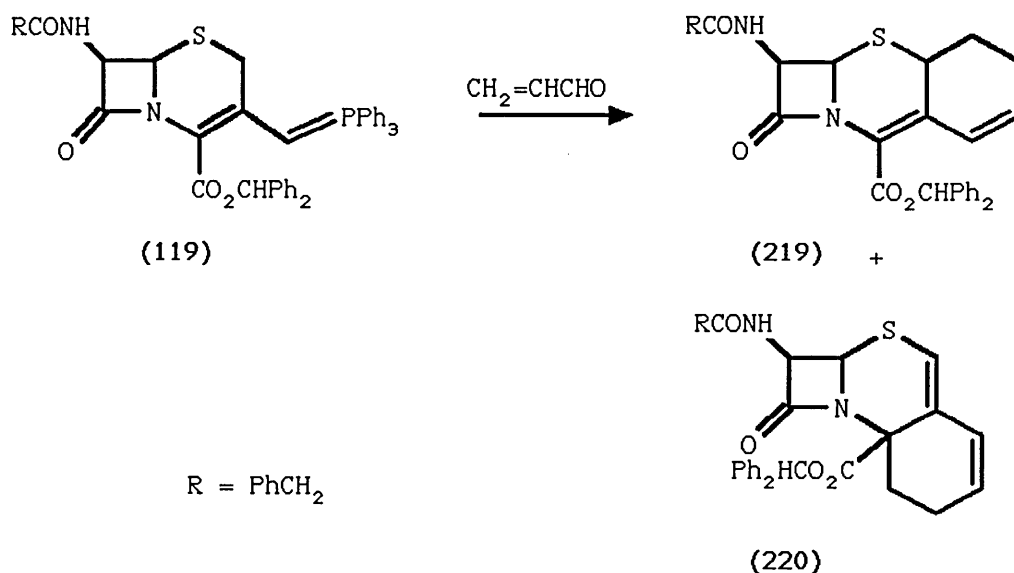
A variety of tricyclic cephalosporin derivatives fused predominantly on the 2,3-positions or the 3,4-positions have been reported¹⁴². Most tricyclic derivatives have been prepared by three main routes. Intramolecular 1,3-cycloaddition reactions have been investigated in a series of papers by Beecham workers, exemplified¹⁴³ by the synthesis of the biologically active tricycle (216) from azetidinone (215). Free-radical cyclisation of



azetidinone (**217**) with Bu_3SnH and AIBN results in formation of the benzocarpacehem (**218**)¹⁴⁴.

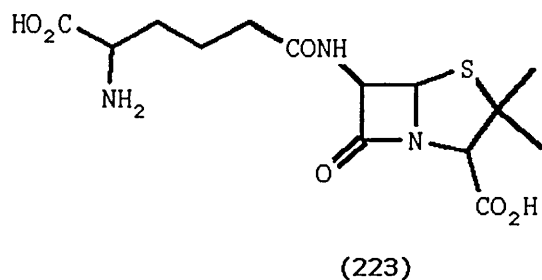
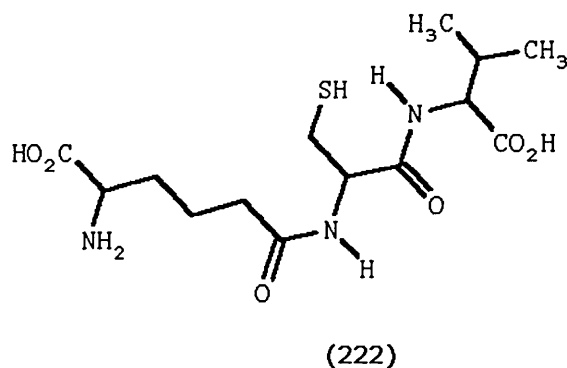
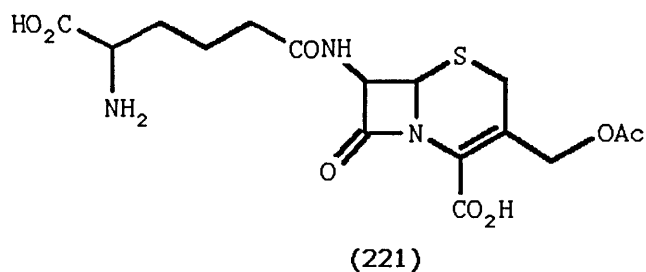


Cephalosporins bearing a C-3' phosphorane (**119**; $\text{R}=\text{PhCH}_2$) react¹⁴⁵ with acrolein, followed by an intramolecular Wittig reaction, to give the tricyclic cepems (**219**) and (**220**).

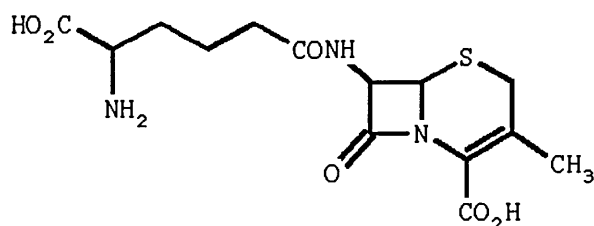


1.7.3) Cephalosporin Biosynthesis

The biosynthesis of cephalosporin C (**221**) is based on the amino acids cysteine, valine and α -aminoadipic acid which combine to form the Arnstein tripeptide¹⁴⁶, α -aminoadipylcysteinylvaline (**222**). This tripeptide has previously been implicated in the biosynthesis of penicillin N (**223**)¹⁴⁷. The organisms which naturally

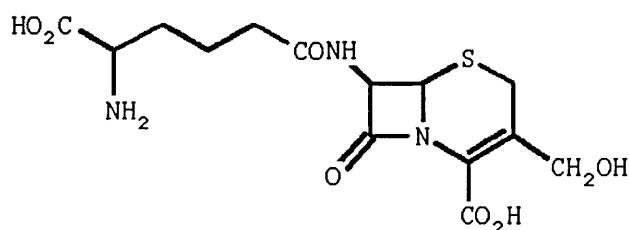


produce cephalosporins which have been most closely studied are *Cephalosporium acremonium* and *Streptomyces clavuligerus*. Since both of these organisms also produce penicillin N, cephalosporin C can be envisaged as being formed directly from tripeptide (222) or via enzymatic ring-expansion of (223). The latter route has been confirmed when it was found¹⁴⁸ that extracts of *C. acremonium* could convert penicillin N into a new antibiotic, deacetoxycephalosporin C (224)¹⁴⁹. Extracts



(224)

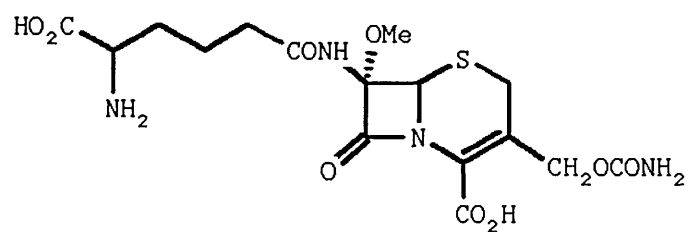
of *S. clavuligerus* have been found to behave similarly¹⁵⁰. Further extracts of both *C. acremonium*¹⁵¹ and *S. clavuligerus*¹⁵² reacted with deacetoxy-cephalosporin C (224) to give a further compound. The enzymic system required molecular oxygen for this conversion, and the 3-hydroxymethylcephem (225) was



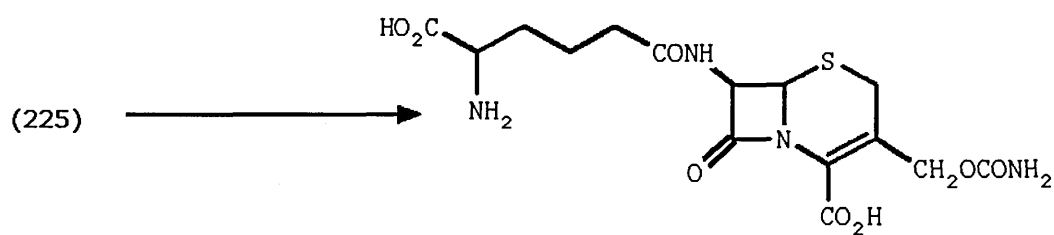
(225)

postulated as the next intermediate in cephalosporin C biosynthesis. This hypothesis was later confirmed by Fujisawa and co-workers¹⁵³. The final stage in the *C. acremonium* biosynthesis is acylation of deacetylcephalosporin C (225)^{151, 154} to give cephalosporin C (221).

In the case of *S. clavuligerus* the cephalosporin produced is cephamycin C (226). Thus, deacetylcephalosporin C (225) is converted into the carbamoyl derivative (227)¹⁵⁵, followed by introduction of the 7 α -methoxy substituent. This final stage is believed to



(226)



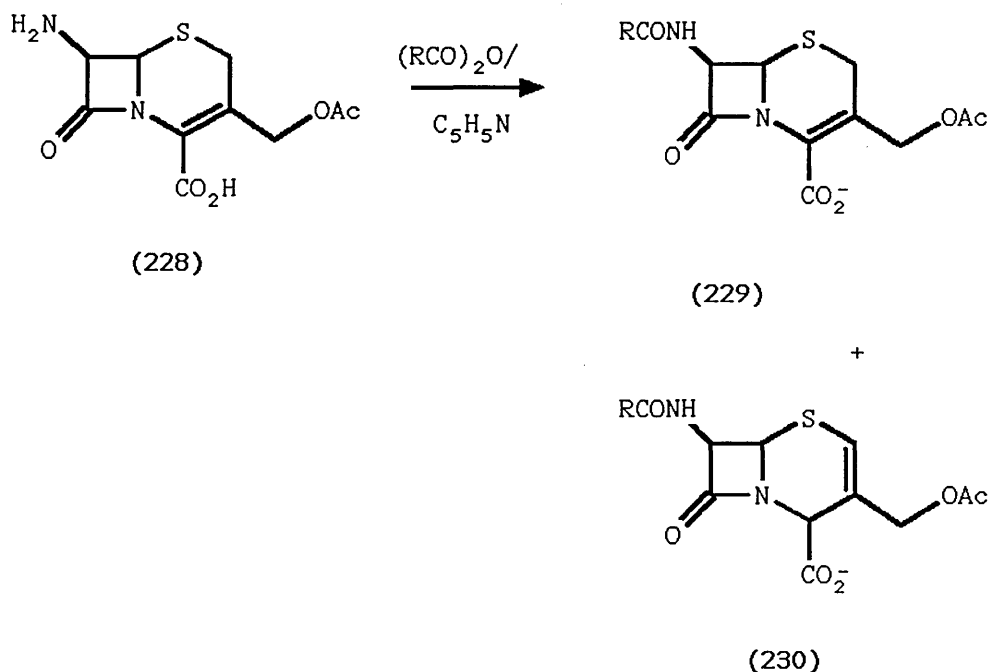
(227)

be a two-step process¹⁵⁶ occurring *via* initial formation of the 7 α -hydroxy derivative, followed by methylation.

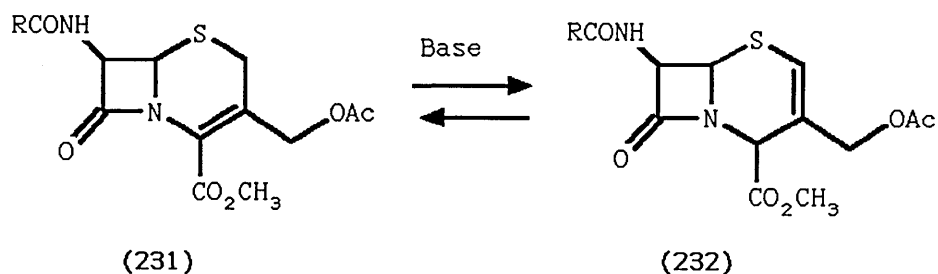
DISCUSSION

2.1) REACTION OF CEPHEMS WITH WEAK ORGANIC BASES.

Whilst investigating the acylation of 7-ACA with acid anhydrides and pyridine, Glaxo workers¹⁵⁷ discovered that, in addition to the expected ceph-3-em system (229), the corresponding ceph-2-em (230) was also obtained. In a separate experiment the acid derived

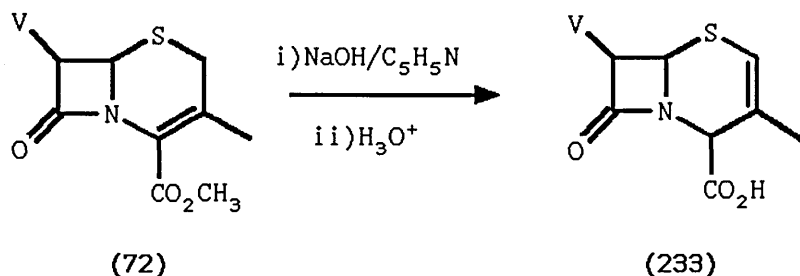


from (229) reacted slowly with pyridine to form (230), whereas methyl esters (231) were readily isomerised by either pyridine or triethylamine to the Δ^2 -cephem (232).

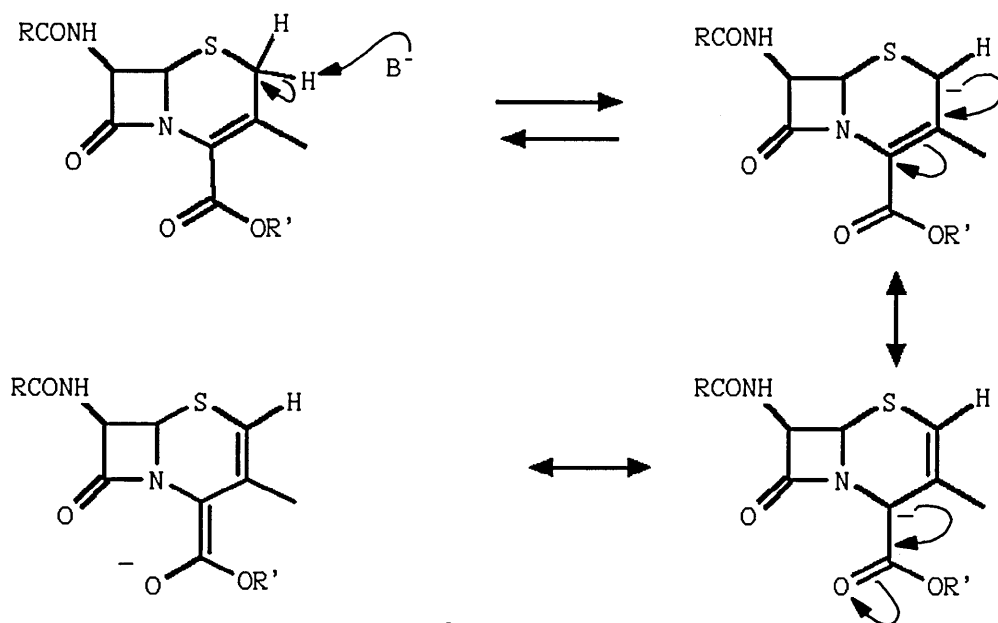


Similarly, Morin *et al*¹⁷ found that methyl ester (72) was converted into the ceph-2-em acid (233) upon hydrolysis of the ester with dilute NaOH in pyridine¹⁵⁸, followed by treatment with dilute acid. Under these

reaction conditions, the ceph-2-em ester hydrolyses to the acid more rapidly, resulting in sole isolation of the ceph-2-em acid after acidification of the reaction mixture. The isomerisation of ceph-3-em esters with



weak organic bases such as triethylamine can be considered to occur as a result of C-2 deprotonation, followed by delocalisation of the negative charge (Scheme 4).

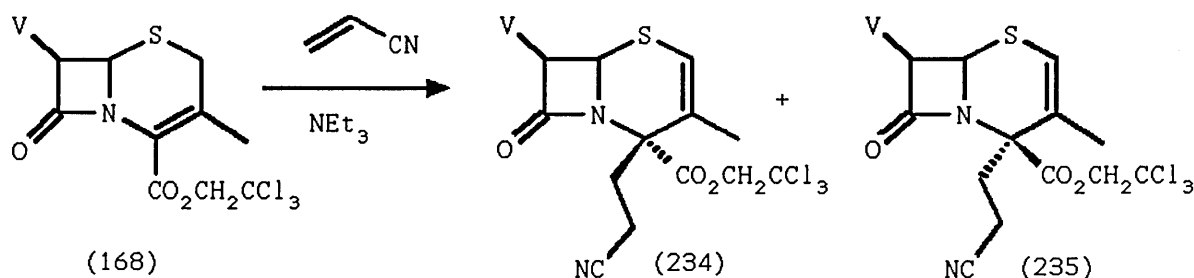


Scheme 4

The possibility therefore exists of trapping these carbanions with suitable electrophiles, leading to C-2 and/or C-4 substitution.

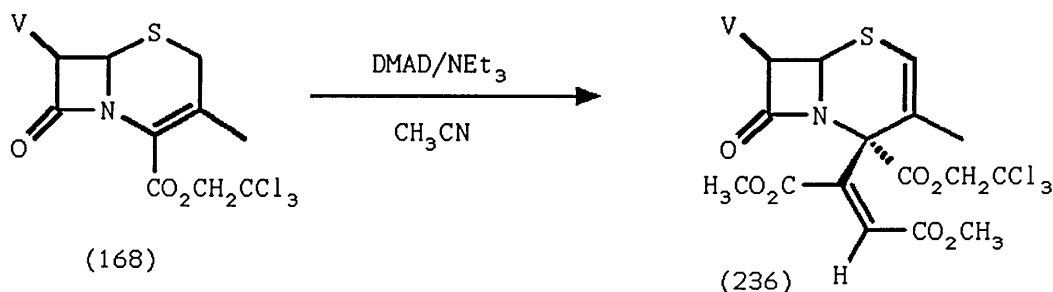
2.1.1) Reaction of Ceph-3-ems with Michael Acceptors.

Bremner and Campbell¹¹⁴ have shown that ceph-3-em (168) reacts with acrylonitrile in the presence of a catalytic amount of triethylamine to give a mixture of 4-disubstituted ceph-2-ems (234) and (235). The major



product (234) was assigned β -stereochemistry on the basis of previous C-4 methylthiolation reactions¹⁵⁹.

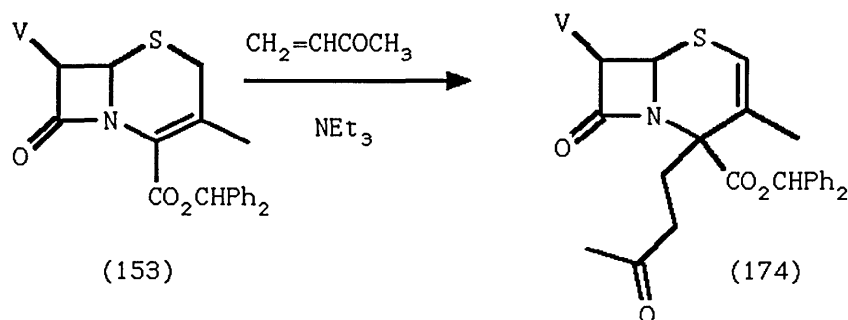
When dimethyl acetylenedicarboxylate was employed as the Michael acceptor, only one product (236) was obtained.



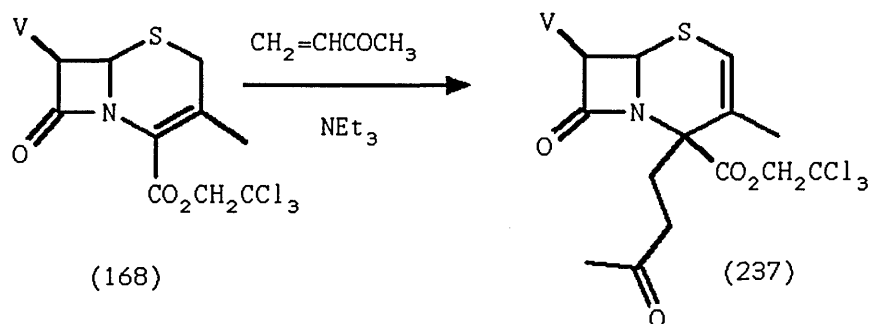
It is apparent from this work that substituted ethyl groups can be incorporated into the 4-position of the cephem nucleus forming C-4 disubstituted ceph-2-ems. In order to extend the scope of this Michael reaction, a variety of alternative Michael acceptors was examined.

When ceph-3-em (153) was stirred at room temperature in methyl vinyl ketone containing a catalytic amount of triethylamine, a single product was obtained in virtually quantitative yield. The pmr spectrum indicated

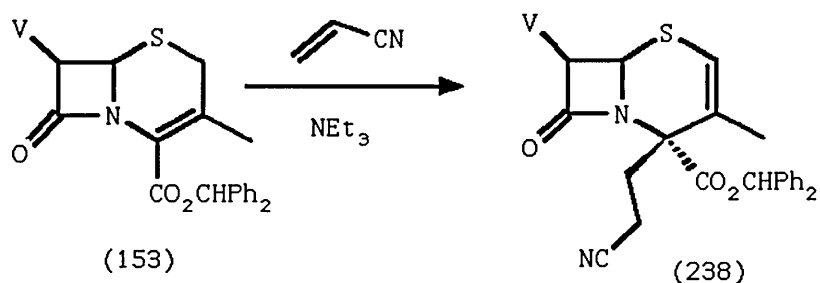
the incorporation of a single methyl vinyl ketone residue with an additional singlet integrating for 3 protons at δ 2.11 and a 4 proton multiplet at δ 2.37-2.93. Vinylic coupling between the C-3 methyl group and the single C-2 proton was observed (1.2Hz), thereby confirming the expected ceph-2-em structure. Elemental analysis and mass spectroscopy data were also consistent with structure (174). Trichloroethyl ester (168) also



reacted under identical conditions to afford ceph-2-em (237) in 51% yield. All analytical and spectroscopic data were consistent with the proposed structure. In

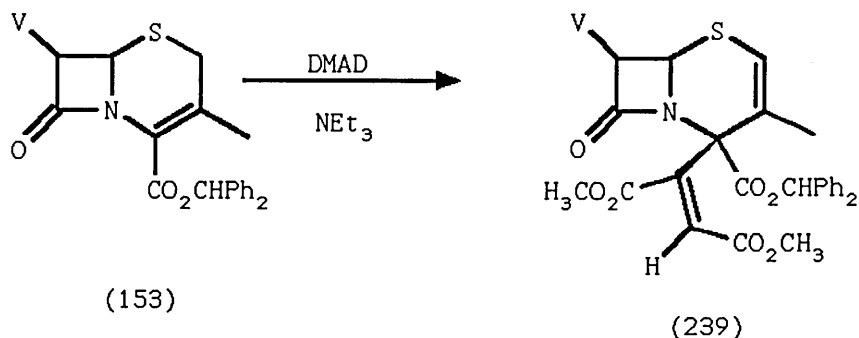


the Michael reaction of these ceph-3-ems with methyl vinyl ketone, the type of C-4 ester has no effect on the course of the reaction. Previously¹¹⁴, when ester (168) was reacted with acrylonitrile, both C-4 isomers (234) and (235) were produced. When the reaction is carried out with diphenylmethyl ester (153), only one isomer is isolated which is assigned β -stereochemistry.



The pmr spectrum of acrylonitrile adduct **(238)** showed vinylic coupling between the H-2 and 3-methyl protons (1.2Hz) and a multiplet for the cyanoethyl group. (Similar coupling between the 3-methyl group and the vinylic C-2 proton had been observed for methyl vinyl ketone adduct **(174)**). A sharp peak at 2248cm^{-1} in the infrared spectrum denoted the presence of the cyano group.

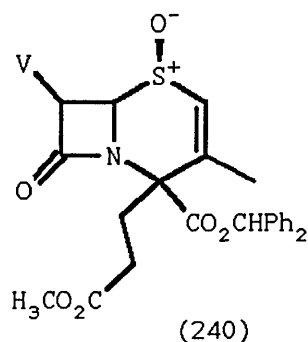
Dimethyl acetylenedicarboxylate also reacted with diphenylmethyl ester **(153)** in an analogous manner to that observed with the corresponding trichloroethyl ester **(168)**. Structure **(239)** is assigned on the basis of literature precedent¹¹⁴, and all spectroscopic and analytical figures are in agreement with the proposed structure.



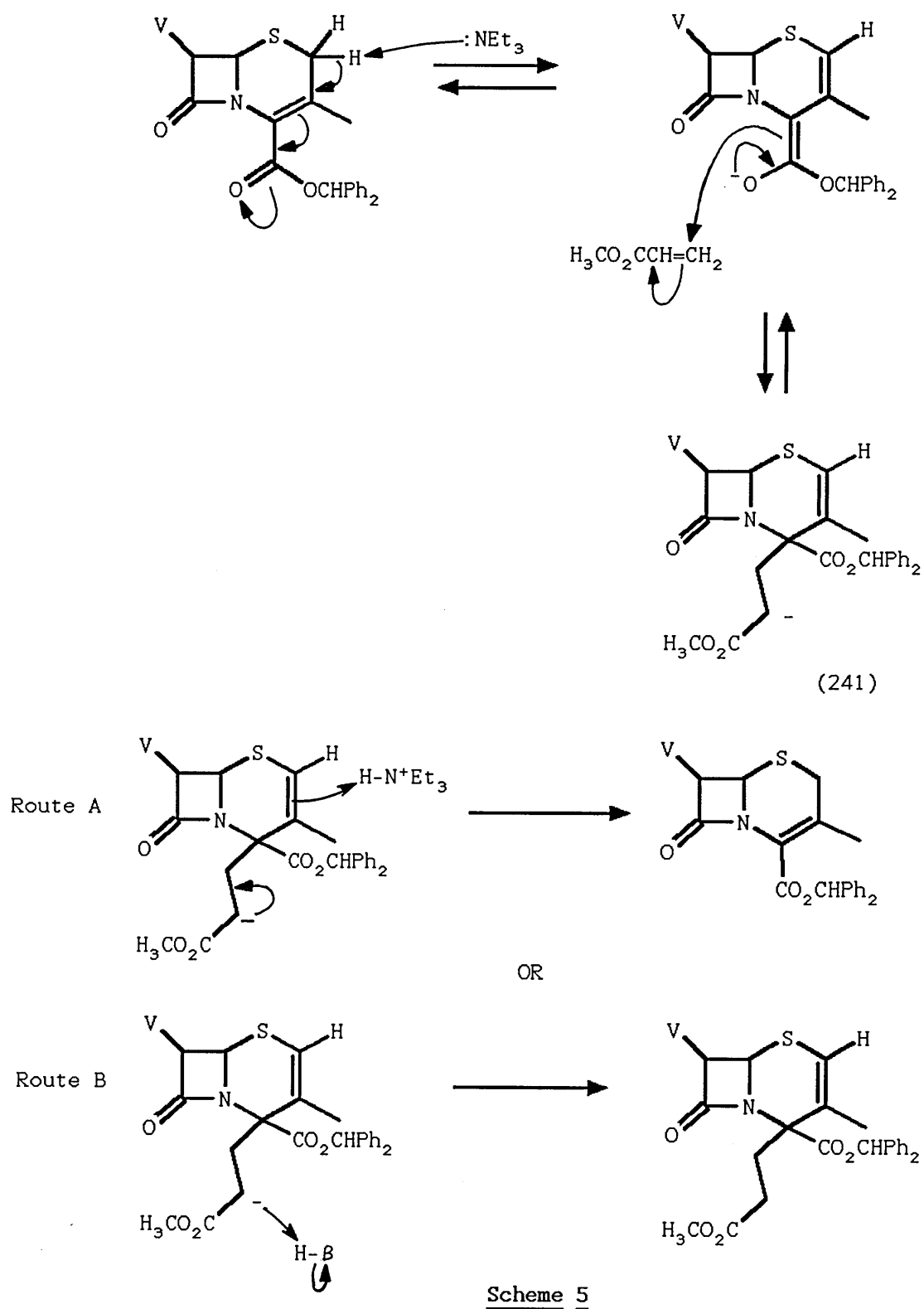
When cephem **(153)** was stirred with methyl acrylate as the Michael acceptor in the presence of triethylamine, no reaction other than Δ -2/ Δ -3 isomerisation was

observed on tlc, even after prolonged reaction times. An aliquot was removed from the reaction mixture and oxidised with *m*-CPBA to ensure that any products did not have a similar R_f to either the Δ -2 or Δ -3 isomers of (153). Analysis of this aliquot showed it to contain one component, with an identical R_f to starting material sulphoxide. Several other bases including pyridine and piperidine were examined but in all cases the sole reaction observed was isomerisation of the double bond. When Triton B (benzyltrimethylammonium hydroxide) was used as base, it initially appeared as though no reaction had occurred due to the presence of a single component on tlc with a similar R_f to starting material. On closer examination of the tlc plate it was apparent that none of the Δ 2-isomer was present, indicating that either (a) Triton B is incapable of causing isomerisation of (153); or (b) that reaction had actually occurred to give a product with an identical R_f to starting material. The former hypothesis was discounted when stirring (153) in dichloromethane containing Triton B gave rise to Δ -2/ Δ -3 isomerisation as previously observed with other organic bases. The crude reaction mixture from the Michael reaction with methyl acrylate was oxidised with *m*-CPBA to give a 2-component mixture which, after chromatography, afforded two amorphous white solids. The minor, more polar component was identical (R_f , mp, pmr) to oxidised starting material. The major product, having a

molecular ion at 616 is consistent with the incorporation of one methoxycarbonyl ethyl group. The pmr spectrum showed the expected vinylic C-2 proton which was coupled to the 3-methyl group ($J=1.2\text{Hz}$) as previously observed. A 3 proton singlet due to the methyl ester and a 4 proton multiplet also indicated (240) as the structure of the product. Analytical data confirmed the molecular formula as $\text{C}_{33}\text{H}_{32}\text{N}_2\text{O}_8\text{S}$.



In the Michael reaction between (153) and methyl acrylate, triethylamine probably fails to catalyse the reaction due to an unfavourable equilibrium. It has been demonstrated above that triethylamine readily catalyses Michael reactions with a variety of acceptors. In the case of methyl acrylate, deprotonation of the cephem followed by alkylation will give rise to the intermediate carbanion (241) as shown in Scheme 5. At this point, protonation can occur via two possible routes to give either recovered starting material or the Michael adduct. With triethylamine, route A is followed, resulting in recovery of starting material. In the presence of Triton B however, route B is preferred and the Michael adduct results. Triton B has

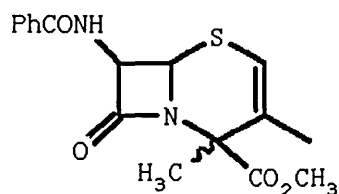


been found to be the catalyst of choice in several condensation reactions including the Michael reaction¹⁶⁰. Additionally, Triton B is supplied commercially as a methanolic solution and the presence of an added proton source will also force the equilibrium toward the product side. It has been noted that the addition of a protic solvent is often beneficial in sluggish Michael reactions¹⁶¹.

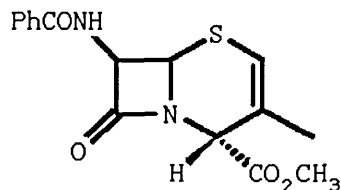
Michael acceptors which completely failed to react under these conditions included acryloyl chloride and dimethyl maleate. In both cases, recovered starting material was obtained quantitatively.

2.1.2) Stereochemistry of Michael Additions.

It has been suggested¹⁶² that alkylation at C-4 in cepheids occurs on the β -face due to repulsion between the N-5 lone pair (on the α -face) and the C-4 carbanion. This repulsion forces the carbanion onto the β -face and so alkylation occurs on the more hindered side of the molecule. PMR studies have shown^{159, 162}, by comparison of the chemical shifts obtained in the presence of lanthanide shift reagents, that alkylation reactions do occur predominantly on the β -face. Thus, the pmr spectrum of ceph-2-em (242) in the presence of varying concentrations of $\text{Eu}[\text{fod}]_3$ was compared to the spectrum of ceph-2-em (243), of known C-4 stereochemistry. The



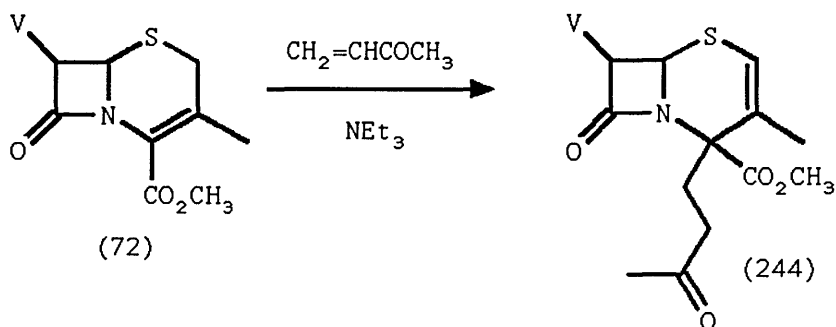
(242)



(243)

signal due to the C-4 ester methyl group in (242) was found to move upfield on increasing concentration of $\text{Eu}[\text{fod}]_3$. In the case of (243) a similar upfield shift was observed confirming the assignment of 4α -carboxyl, 4β -methyl stereochemistry in (242).

It has been assumed¹¹⁴ that C-4 Michael adducts also have 4α -carboxyl, 4β -alkyl stereochemistry. In order to confirm this assumption Michael adduct (244) was prepared from methyl ester (72) by reaction with methyl vinyl ketone and triethylamine. Only one of the two



possible C-4 isomers was obtained and all spectroscopic and analytical data for (244) were in agreement with the proposed structure.

When the pmr spectrum of (244) was recorded in the presence of 0.2 molar equivalents of $\text{Eu}[\text{fod}]_3$, the 3 proton singlet due to the methyl ester experienced a decrease in chemical shift from $\delta 3.78$ to $\delta 3.74$. Increasing the concentration of the shift reagent

resulted in a decrease in δ value of the methyl ester protons as shown in Table 1.

Concentration of Eu[fod] ₃ as molar equivalents of (244)	δ
0	3.78
0.2	3.74
0.4	3.69
0.6	3.65
0.8	3.63
1.0	3.62

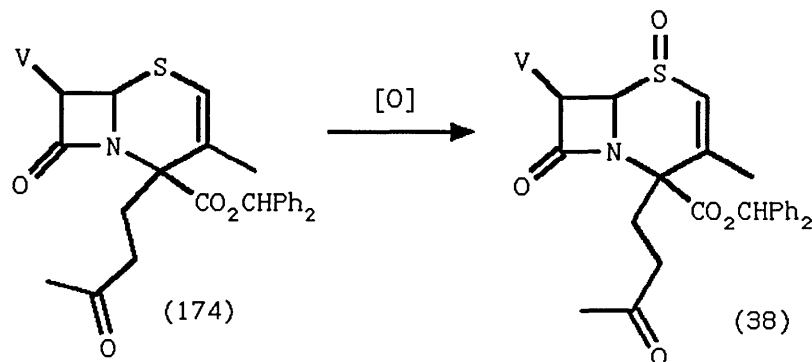
Table 1

This behaviour is identical to that observed by Oida *et al*¹⁶² for C-4 disubstituted ceph-2-ems (242) and (243). The Eu[fod]₃ induced shift of the methyl ester protons in (242) at 1.0 mole equivalent of Eu[fod]₃ was -0.55 δ .

2.1.3) Oxidation of Cephem Michael Adducts.

It has been shown¹⁶³ that, in general, unsaturated sulphoxides adopt the β,γ -configuration in preference to α,β -unsaturation. A similar preference for β,γ unsaturation in cephem sulphoxides has also been shown to exist⁴³. Thus, oxidation of a ceph-2-em or ceph-3-em system results in formation of the ceph-3-em sulphoxide exclusively. In the case of ceph-2-em systems such as (174), no possibility of double bond isomerisation during oxidation exists, therefore the α,β -unsaturated sulphoxide (38) is produced. It was anticipated that these C-4 disubstituted ceph-2-em sulphoxides would

undergo novel reactions as a direct consequence of their being fixed in an unusual stereochemistry.

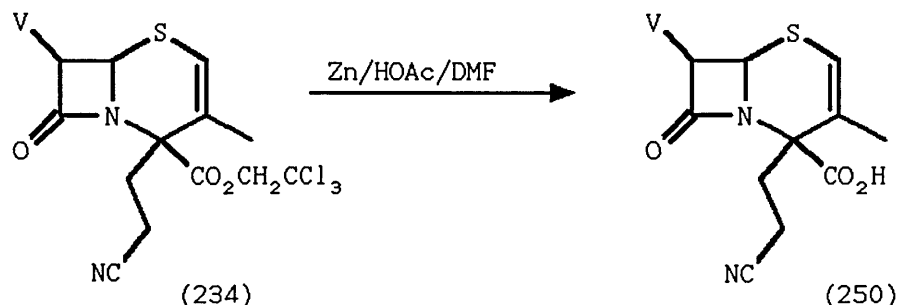


Oxidation of cephem sulphides containing 7 β -amide side-chains with peracids gives rise to the β -sulphoxide as the sole product³⁰. This has been explained *via* 'reagent approach control'³⁰ whereby the incoming oxidant molecule forms a hydrogen bond with the 7 β -amide proton resulting in oxidation on the β -face.

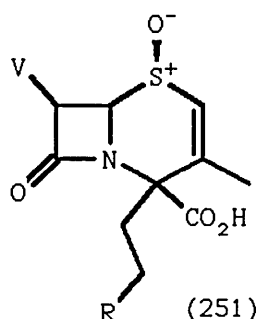
When ceph-2-em (174) was oxidised with *m*-CPBA in dichloromethane at 0°C, only one component was observed on tlc. The pmr spectrum of the isolated product showed retention of the ceph-2-em system, as evidenced by the vinylic C-2 proton (δ 6.71) coupled with the C-3 methyl group ($J=0.9\text{Hz}$). Oxidation to β -sulphoxides has been shown¹⁶⁴ to result in a downfield shift of the H-7 and NH signals and an upfield shift of the 6-H signal when the spectrum is recorded in CDCl_3 . Similar shifts were observed in this case, confirming the β -orientation of the sulphoxide group in (38). Mass spectroscopy

2.1.4) Formation of C-4 Monosubstituted Ceph-3-ems from Michael Adducts.

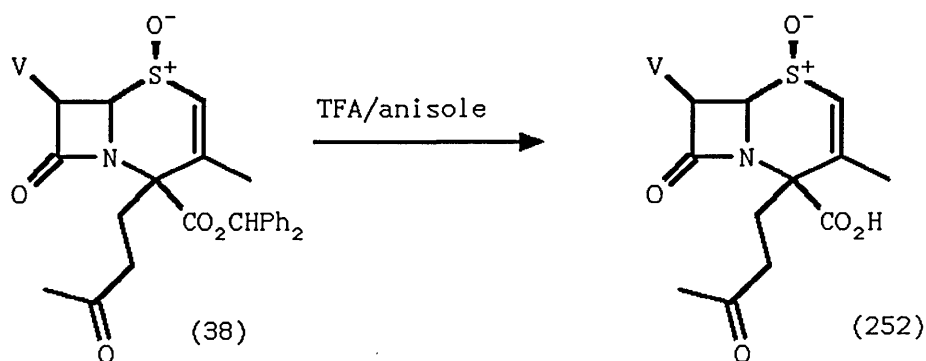
De-esterification of Michael adduct (234) with Zn/AcOH in DMF¹⁶⁵ afforded the ceph-2-em carboxylic acid (250)



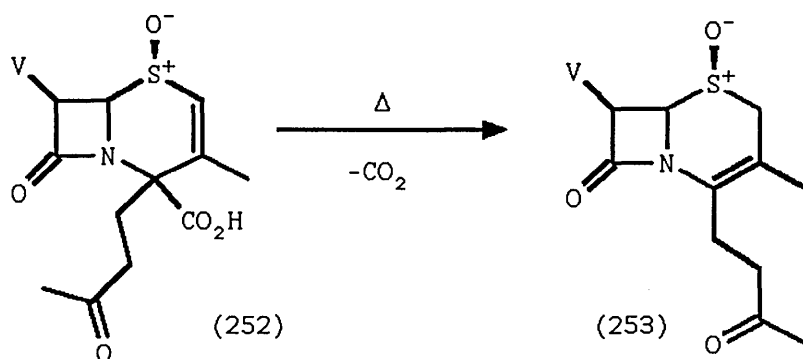
which did not show any antimicrobial activity¹¹⁴. In order to ascertain whether C-4 disubstituted sulfoxide acids of type (251) exhibited any biological activity,



ester (38) was stirred with trifluoroacetic acid in anisole at room temperature for 30 minutes. TLC showed conversion to a very polar component which would only move in BuOH/EtOH/H₂O (4:1:5). Isolation of the crude product, which was assumed to be the acid (252), from the reaction gave a yellow solid which had peaks at 3060-2580, 1778 and 1752cm⁻¹ in the infrared spectrum. This indicated the presence of a carboxylic acid group

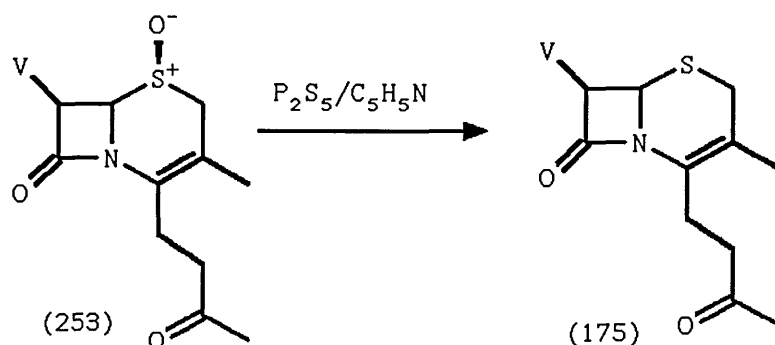


and a β -lactam carbonyl. Attempted recrystallisation of the crude solid from refluxing acetone gave a white powder which, when examined on tlc, was found to be very much less polar than the crude product obtained from the de-esterification. The infrared spectrum of this product showed the continued presence of the β -lactam carbonyl (1761cm^{-1}) but the OH stretch and C=O stretch of the C-4 carboxylic acid function were no longer present. The pmr spectrum showed no downfield protons due to the carboxylic acid. Additionally, the C-2 vinyl proton observed in (38) at $\delta 6.72$ had disappeared, and an AB quartet ($\delta 3.15$ & 3.43 , $J=18.0\text{Hz}$) indicated the presence of two C-2 protons in a ceph-3-em system. Long-range coupling ($J=1.2\text{Hz}$) between H-6 and the more upfield doublet of the C-2 AB quartet was observed. Previously this coupling has only been observed in ceph-3-em β -sulfoxides between H-6 α and H-2 α^{164} , again indicating that the C-2 proton is no longer vinylic. The loss of the C-4 acid function, taken in conjunction with the formation of a Δ -3 double bond system, suggests that thermal decarboxylation of acid (252) had occurred to give the C-4 monosubstituted ceph-3-em (253). Mass



spectroscopy and elemental analysis both confirmed the structure of **(253)**.

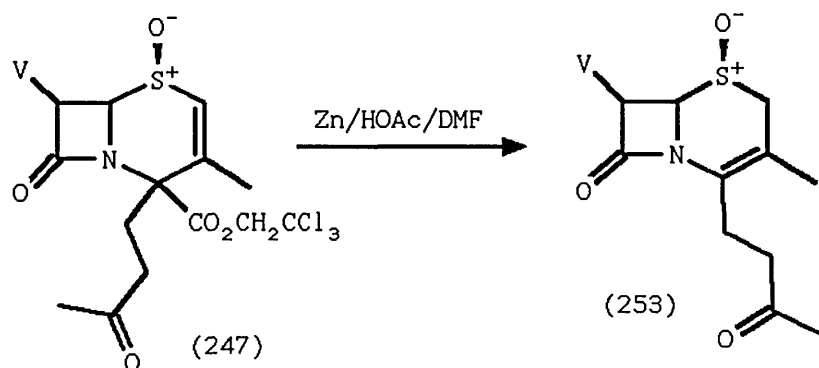
To complete the reaction sequence, sulphoxide **(253)** was de-oxygenated with $\text{P}_2\text{S}_5/\text{pyridine}$ ⁴⁴ to afford the corresponding sulphide **(175)**. No long-range coupling between H-2 α and H-6 α was evident in the pmr spectrum, although an AB quartet was still present for the C-2 protons. Elemental analysis confirmed the molecular formula of **(175)** as $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_4\text{S}$. The overall yield of



compound **(175)** from ceph-3-em **(153)** is 37%.

In the case of the corresponding trichloroethyl ester **(247)**, de-esterification with zinc and acetic acid in DMF resulted in a polar material with a similar R_f to **(252)**. After working-up the reaction mixture however, the only product visible on tlc had an identical R_f to

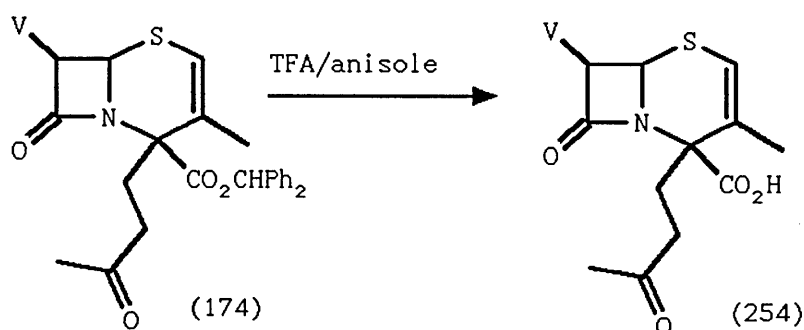
decarboxylated ceph-3-em (**253**). Further analysis of the



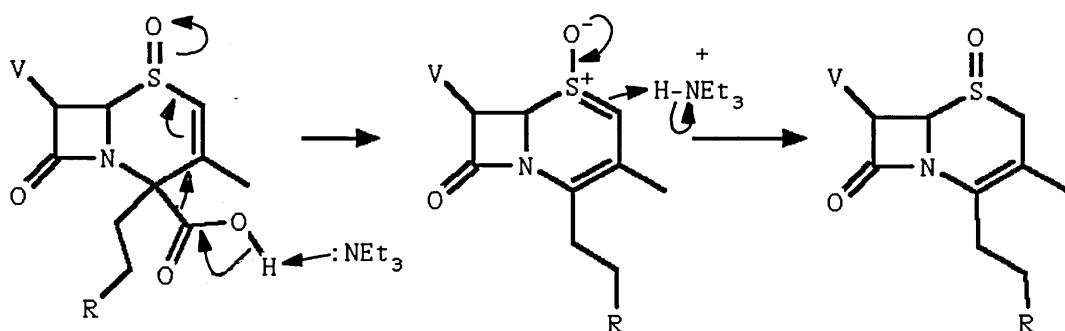
product indicated that it was identical (mp,pmr) to (**253**) and in this case base-induced decarboxylation of the acid must occur when the reaction mixture is washed with NaHCO₃ solution to remove excess acetic acid. Thus, decarboxylation of ceph-2-em C-4 carboxylic acids such as (**252**) can occur either thermally or in the presence of base. Weak organic bases were also found to induce decarboxylation. Thus, when ester (**38**) was de-esterified with TFA/anisole followed by treatment of the crude acid with NEt₃, ceph-3-em (**253**) was obtained in 79% yield.

Decarboxylation was not previously observed when the ceph-2-em sulphide (**234**) was de-esterified with Zn/HOAc¹¹⁴. In order to ascertain whether the sulfoxide function was a necessary requirement for decarboxylation, the ceph-2-em sulphide (**174**) was de-esterified with TFA/anisole to give a very polar product, as observed on tlc. Isolation of this compound resulted in a white foam which exhibited a broad O-H stretch (3700-3000cm⁻¹) in the infrared spectrum in addition to the expected β -lactam carbonyl stretch. The

crude foam was crystallised from ethyl acetate/dichloromethane to afford a white solid which was identical on tlc to the major component in the crude foam. The pmr spectrum indicated loss of the diphenylmethyl group, however no downfield proton could be detected for the resultant C-4 carboxylic acid. The C-2 vinyl proton (δ 6.38) indicated retention of the ceph-2-em structure, but on this occasion no coupling was observed with the 3-methyl group. On the basis of these data, along with mass spectroscopy and elemental analysis, structure (254) is assigned to the acid. This

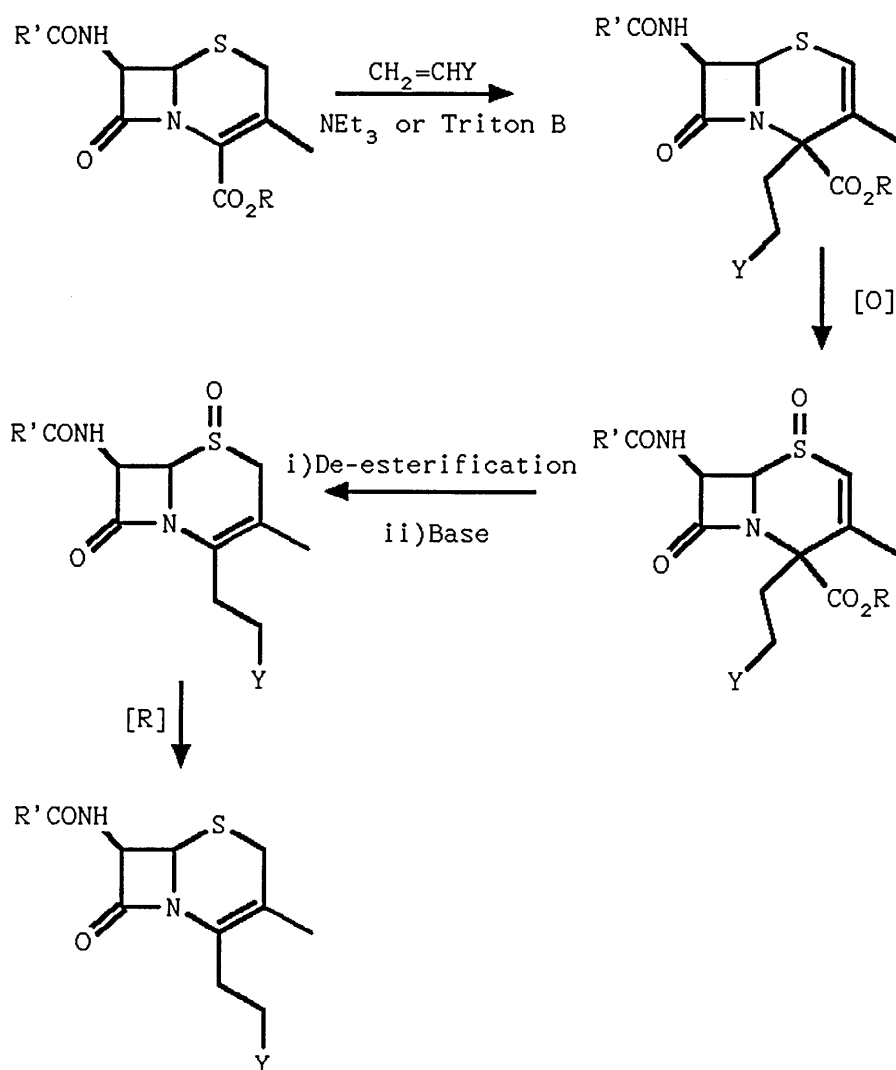


acid was found to be stable to both thermal and base-induced decarboxylation. Treatment of (254) with NEt_3 over an extended time period showed no reaction, with the acid being recovered in quantitative yield. Thus, it is necessary to have the sulfoxide function present in these simple adducts for decarboxylation to occur and the proposed mechanism is shown in Scheme 6.



Scheme 6

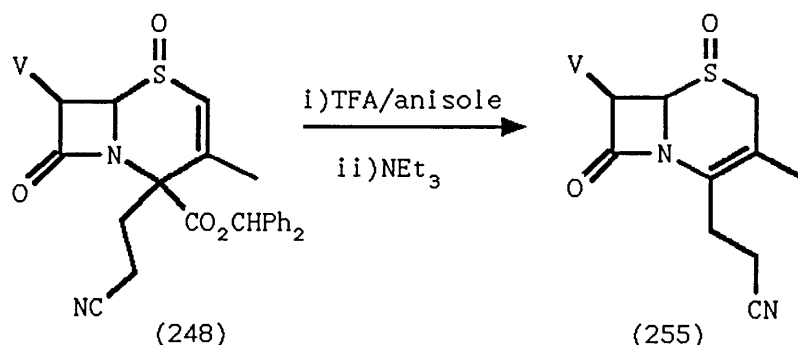
A route to C-4 monosubstituted ceph-3-ems with the original C-4 carboxyl group replaced by a substituted ethyl group was now apparent (Scheme 7), and the scope



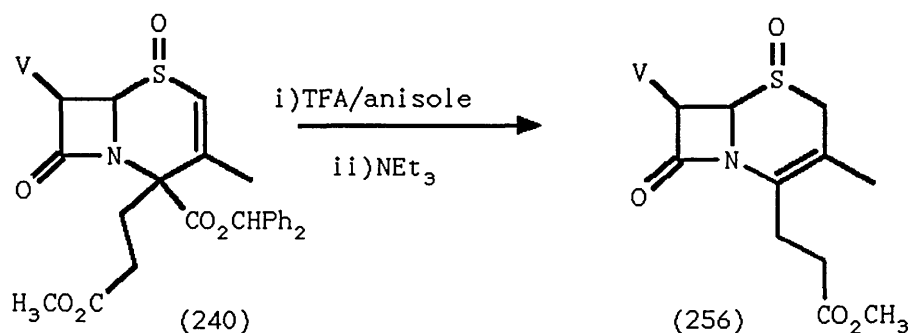
Scheme 7

and limitations of this route were examined utilising other Michael adducts. When acrylonitrile adduct (**248**) was stirred with trifluoroacetic acid and anisole for 30 minutes, complete conversion of the starting material to a more polar component was observed on tlc.

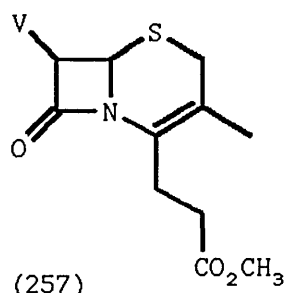
Decarboxylation of this crude acid with triethylamine afforded a less polar product which showed peaks at 2246, 1765 and 1685 cm^{-1} in the infrared spectrum corresponding to the C-4 cyano group, β -lactam carbonyl and side-chain amide carbonyl. The pmr spectrum indicated loss of the diphenylmethyl group and a Δ -3 double bond was found to be present by the lack of a vinylic C-2 proton and the presence of an AB quartet due to the two C-2 protons of a ceph-3-em. Elemental analysis and mass spectroscopy data also indicated the formation of ceph-3-em (**255**). In an analogous manner,



methyl acrylate adduct (**240**) was treated with TFA/anisole followed by reaction of the crude product with triethylamine to afford methyl ester (**256**).

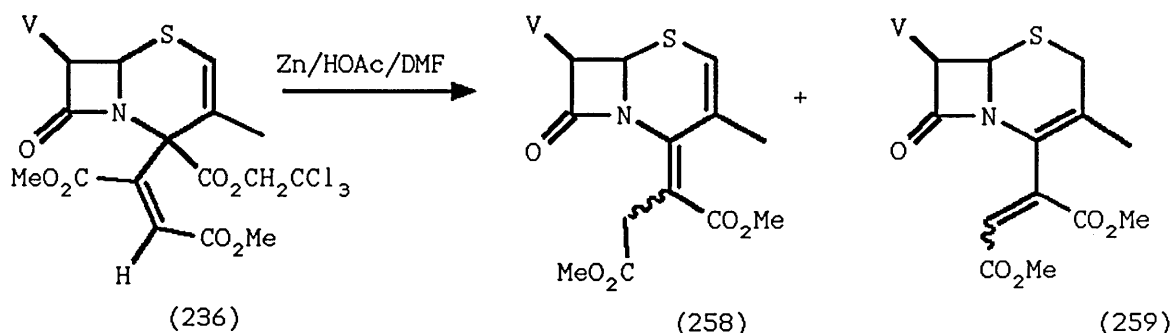


De-oxygenation of sulfoxide **(256)** with P_2S_5 /pyridine resulted in formation of the corresponding sulphide **(257)**, giving an overall yield from ceph-3-em **(153)** to ester **(257)** of 26%.

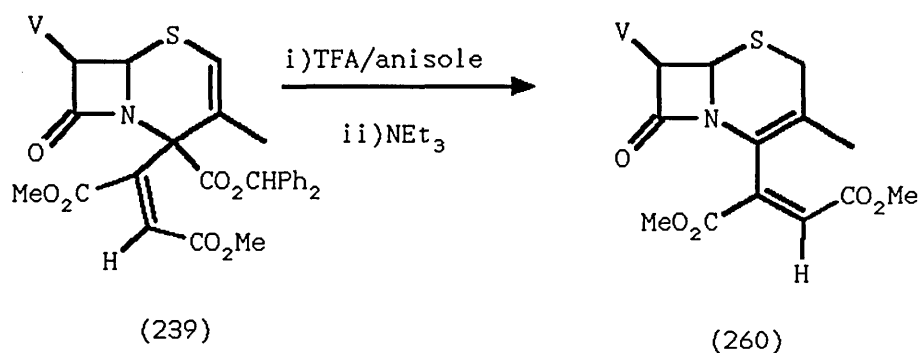


In the case of dimethyl acetylenedicarboxylate adducts **(246)** and **(249)**, de-esterification was found to be somewhat more complex. Reaction of diphenylmethyl ester sulfoxide **(249)** with TFA/anisole followed by treatment of the crude product with NEt_3 resulted in a complex reaction mixture as observed on tlc. Column chromatography appeared to afford a major component, however the pmr spectrum indicated the presence of at least 3 compounds. It did not prove possible to isolate any pure material from the reaction mixture, even after extensive chromatography. Bremner & Campbell found¹¹⁴ that de-esterification of triester **(236)** with Zn/AcOH in DMF resulted in two pairs of isomers **(258)** and **(259)**

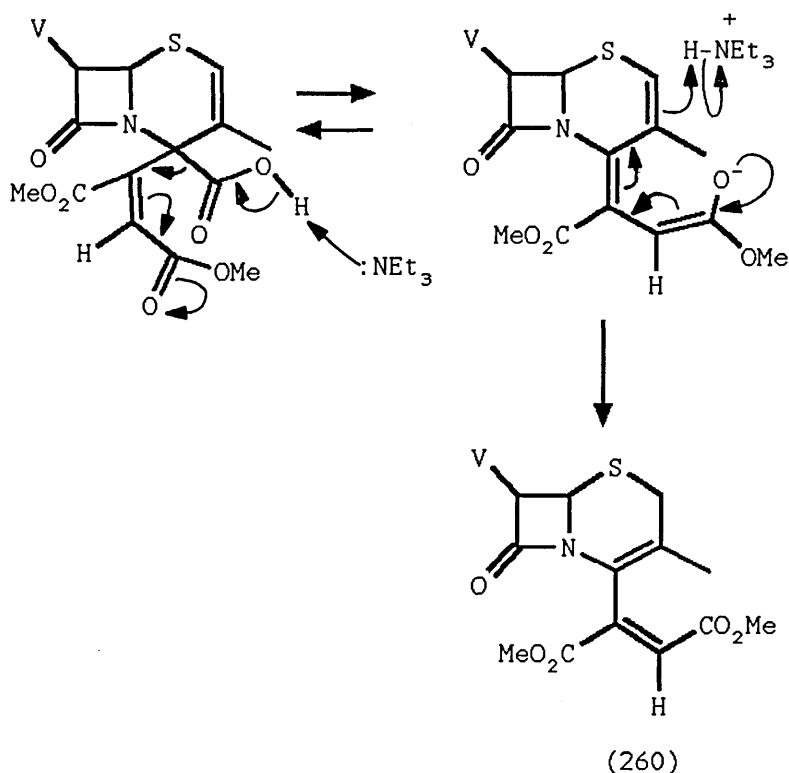
rather than the expected C-4 acid. It appears likely



that attempts to de-esterify and decarboxylate (249) result in similar products (though as the respective sulphoxides) which could not be separated. As discussed above, decarboxylation of C-4 disubstituted carboxylic acids such as (252) can only occur when a sulphoxide function is present. However, when sulphide (239) was reacted with TFA/anisole the crude product obtained was found to react in the presence of triethylamine to form a component which was less polar than the crude acid. The infrared spectrum indicated loss of the C-4 carboxyl group although the β -lactam carbonyl was still present. The C-2 protons existed in the pmr spectrum as an AB quartet indicating a cep-3-em structure. The unsaturated diester was evidenced by the presence of two 3 proton singlets for the methyl esters and a proton at δ 6.32 due to the vinylic fumarate proton. Thus, structure (260) is assigned which is also in agreement with the observed molecular formula and molecular weight.



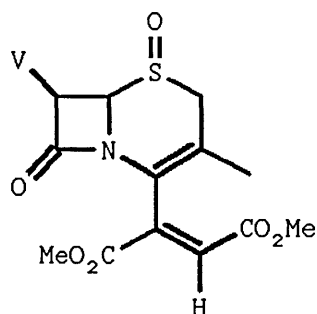
In this instance, decarboxylation can occur in the absence of the sulfoxide function *via* delocalisation of the negative charge into the vinylogous β -diester as shown in Scheme 8.



Scheme 8

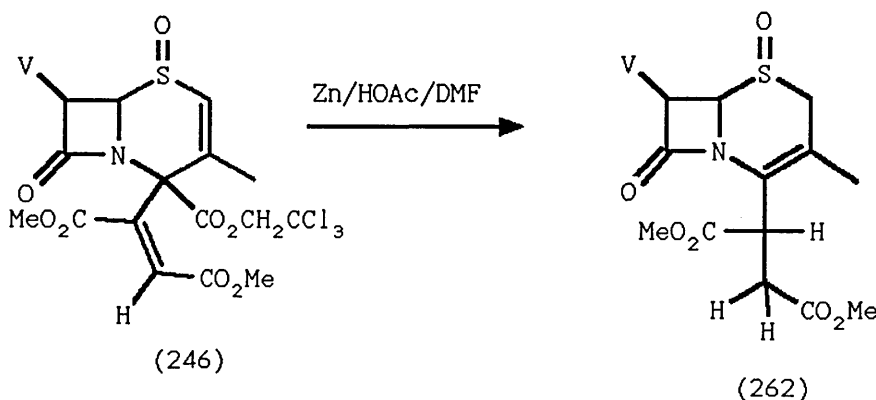
In the corresponding trichloroethyl ester series, de-esterification of (246) with zinc in acetic acid/DMF gave a single product as a white crystalline solid. The pmr spectrum showed loss of the trichloroethyl group and a Δ -3 double bond was indicated by the presence of an AB

quartet for the two C-2 protons. However, the vinyl proton previously seen in the fumarate double bond system of (246) at $\delta 6.46$, was no longer present and 3 further protons at $\delta 2.78$, $\delta 3.25$ and $\delta 4.36$ were observed. Elemental analysis indicated a molecular formula of $C_{21}H_{24}N_2O_8S$, which was supported by a molecular ion at 464; thus 2 additional protons were present compared to the expected product (261).



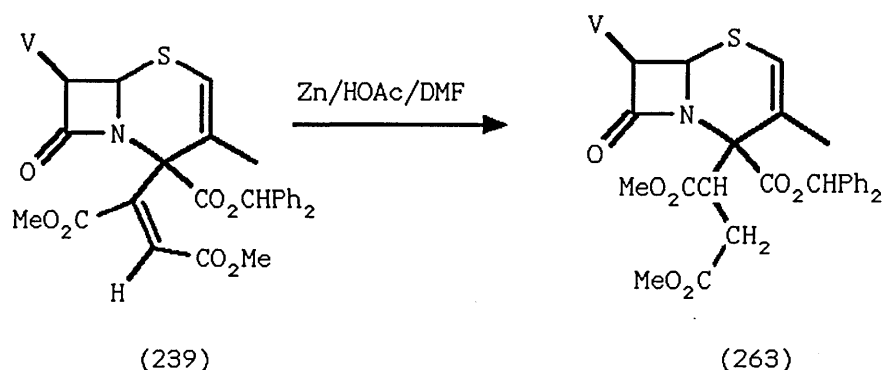
(261)

Zinc/acetic acid is a well known reducing agent, and reduction of unsaturated systems to the corresponding saturated system has been reported in a variety of cases¹⁶⁶. In the reaction above, the α,β -unsaturated diester system is also susceptible to reduction, hence the product is the saturated diester (262), the structure of which is in agreement with all spectroscopic and analytical data. Attempts to

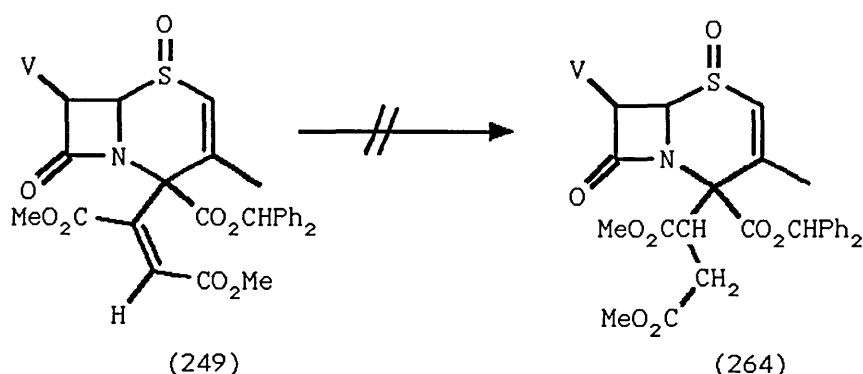


de-oxygenate (262) using P_2S_5 /pyridine resulted in a complex mixture from which no pure compounds could be isolated even after extensive chromatography. The use of KI/CH_3COCl gave rise to a similarly complex mixture. The pmr spectrum and mass spectrum of the crude reaction mixtures were of no assistance in trying to identify any products.

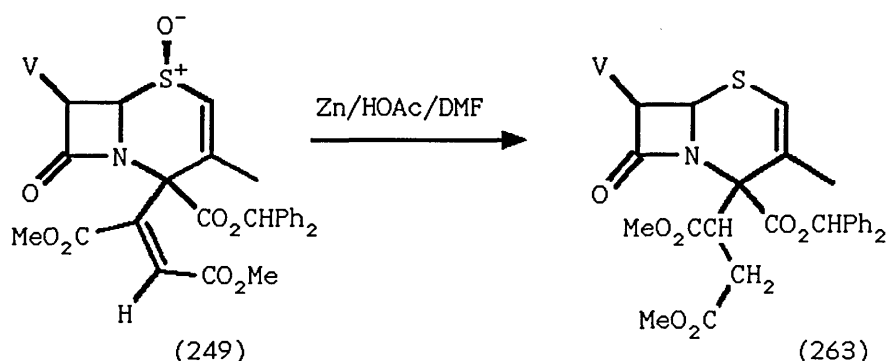
To confirm that the unsaturated diester system in (246) was susceptible to reduction when the ceph-2-em structure was fixed (ie: that reduction of the diester was not occurring solely as a consequence of initial de-esterification and decarboxylation), diphenylmethyl ester (239) was reacted with Zn in AcOH/DMF. The pmr spectrum of the single product obtained from the reaction showed the expected vinylic coupling between the C-2 proton and the C-3 methyl group characteristic of a ceph-2-em. Three protons with chemical shifts and coupling constants indicative of the disubstituted ethyl group were observed. Elemental analysis and mass spectroscopy also suggested reduction of the unsaturated diester. Therefore, structure (263) is assigned to the product from this reaction. When the corresponding



sulphoxide (249) was reacted under the same conditions, a single product was isolated which had an identical R_f to (263). The pmr spectrum again showed a ceph-2-em double bond and the 3 protons of the saturated diester system were also observed. The chemical shift values for the H-6, H-7 and N-H signals did not show any marked difference from those in the sulphide (263) which is surprising in light of the shifts observed in these signals in other β -sulphoxides¹⁶⁴. Mass spectroscopy indicated a molecular weight of 658 which was 16 less than the expected weight of sulphoxide (264). Elemental analysis confirmed the molecular formula as $C_{35}H_{34}N_2O_9S$ and hence an oxygen has been lost from the expected product (264). Thus, in the presence of zinc and acetic



acid, sulphoxide (249) not only undergoes reduction of the unsaturated diester as expected, but the sulphoxide function is also reduced to give sulphide (263).

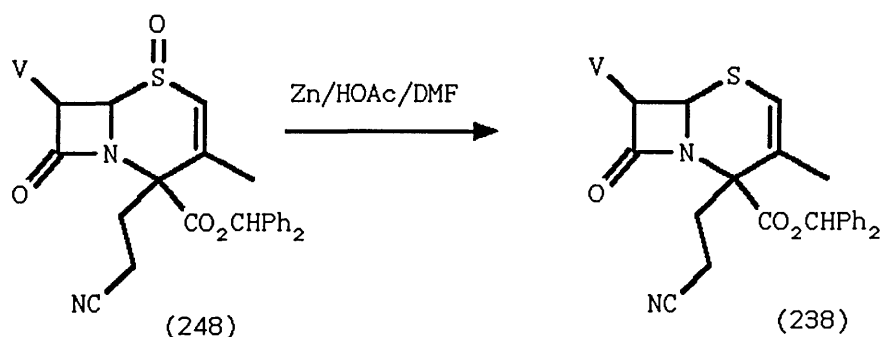


This mild de-oxygenation of ceph-2-em sulfoxides contrasts markedly with the relative difficulty experienced in the de-oxygenation of ceph-3-em sulfoxides which normally require the presence of an activating agent in addition to a reducing agent⁴³.

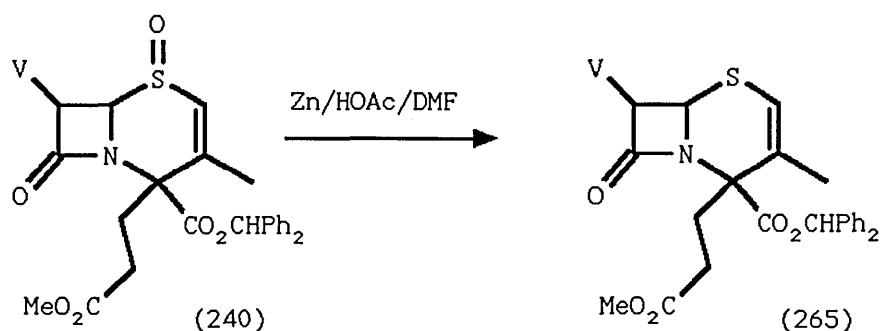
2.1.5) De-oxygenation of Ceph-2-em Sulfoxides.

The discovery that ceph-2-em sulfoxide (249) underwent de-oxygenation to the sulphide under mild conditions prompted further investigation of the de-oxygenation of ceph-2-em sulfoxides.

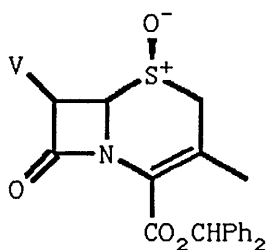
When acrylonitrile adduct sulfoxide (248) was stirred with zinc dust in acetic acid/DMF formation of a considerably less polar component was observed on tlc and after 3 hours no starting material remained. Neutralisation and work-up of the reaction mixture afforded a single product in high yield (97%). The pmr spectrum was similar to that of (248), however shifts of +0.628 in the H-6 signal and -0.398 in the H-7 signal were observed¹⁶⁴, suggesting that de-oxygenation had occurred to give compound (238). Both mass spectroscopy and elemental analysis were in accordance with this proposed structure. In a similar manner, methyl



acrylate adduct (240) de-oxygenated in the presence of zinc/acetic acid in DMF to give sulphide (265). All analytical and spectroscopic data were consistent with the proposed structure.

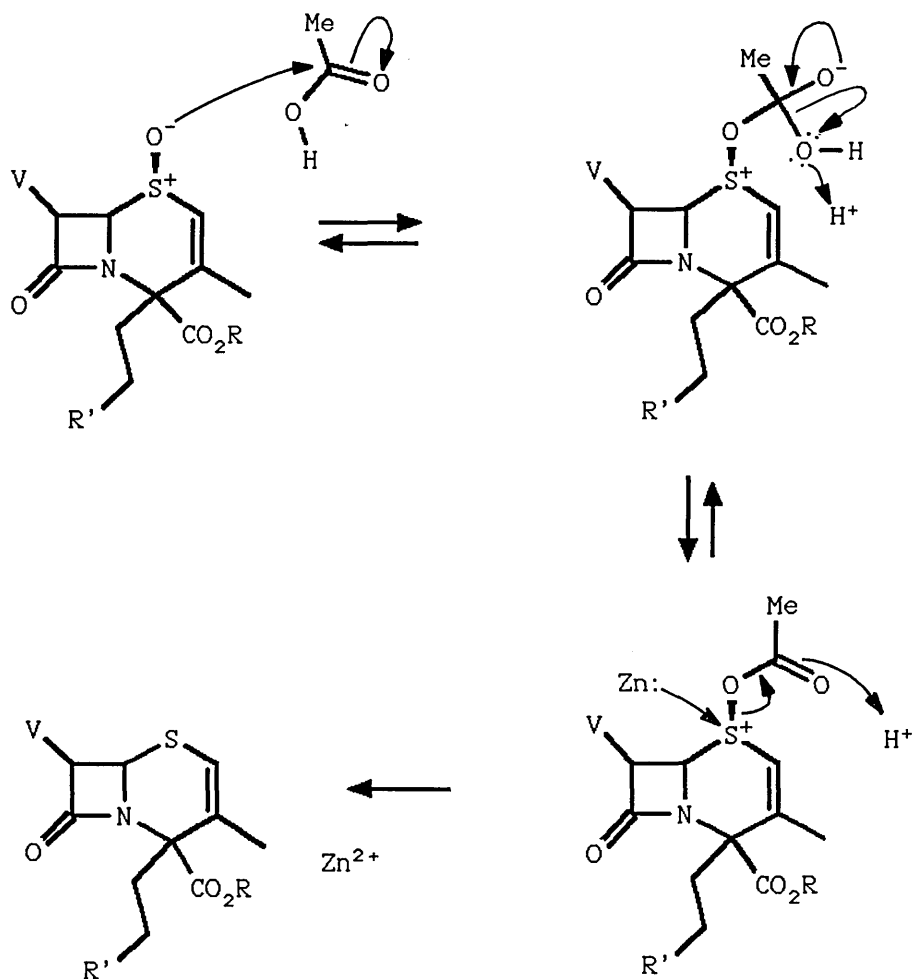


With trichloroethyl ester sulfoxides such as (247), reaction with zinc/acetic acid can give rise to de-esterification and/or de-oxygenation of the sulfoxide. It has been shown above that reaction of (247) with Zn/AcOH gives the ceph-3-em sulfoxide (253) with no evidence of de-oxygenation. Thus, it is proposed that with trichloroethyl esters, de-esterification of the ester followed by spontaneous decarboxylation of the ensuing acid results in a ceph-3-em sulfoxide which will not de-oxygenate under these mild conditions. The failure of ceph-3-em sulfoxides to de-oxygenate with zinc/acetic acid was verified by stirring sulfoxide (266) for several days



(266)

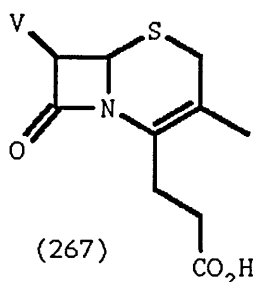
under conditions which caused de-oxygenation in ceph-2-em sulfoxides. No reaction was observed and starting material was recovered in quantitative yield. The difficulty associated in reducing ceph-3-em sulfoxides has been attributed to electronic effects present in the Δ -3 sulfoxide⁴³. Primarily the electron-withdrawing effect of the β -lactam nitrogen and the C-4 carboxyl group combine to make the sulfoxide bond stronger than a normal aliphatic sulfoxide. In the ceph-2-em sulfoxide such electronic effects are decreased allowing de-oxygenation to occur under more facile conditions. The proposed mechanism for this de-oxygenation is shown in Scheme 9.



Scheme 9

2.1.6) Carboxylic Acids Prepared from C-4 Michael Adducts.

The development of the scheme detailed above whereby a ceph-3-em ester could be converted into a ceph-3-em with the original carboxylate group replaced by a substituted ethyl group had obvious synthetic possibilities. In particular, the introduction of a propanoic acid residue into the cephem nucleus at C-4 would give rise to the novel cephem acid (267).



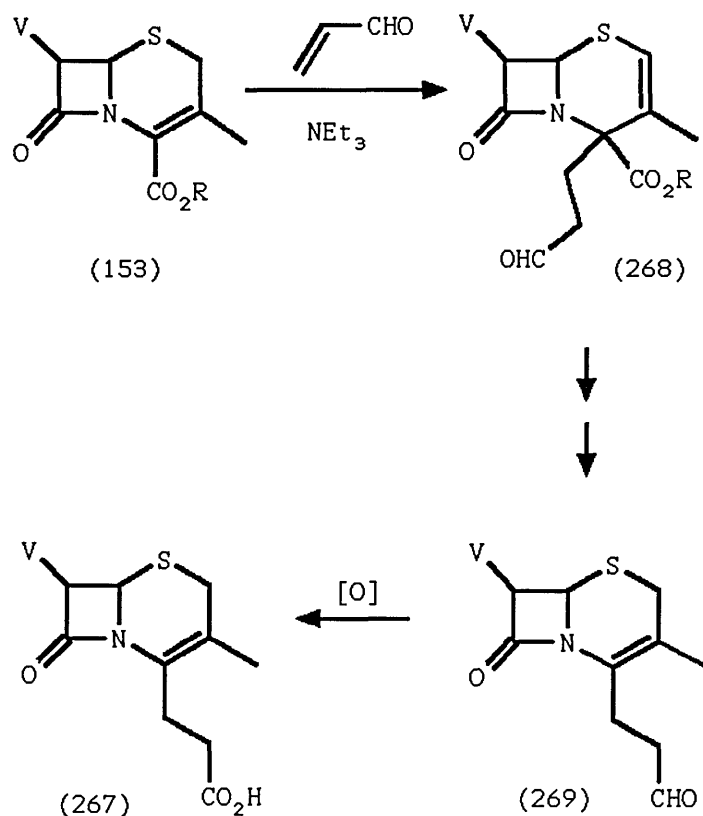
Since methyl acrylate reacted with ceph-3-ems in the presence of Triton B, the most direct route to acid (267) was envisaged as being *via* a Michael reaction with an ester of acrylic acid which could be easily hydrolysed. Esterification of acrylic acid with trichloroethanol, catalysed by concentrated H_2SO_4 , resulted in formation of trichloroethyl acrylate in 37% yield. When ceph-3-em (153) was reacted with trichloroethyl acrylate under identical conditions to those employed with methyl acrylate, only Δ -3/ Δ -2 isomerisation was observed (by tlc). Use of triethylamine as base also failed to achieve incorporation of the acrylate residue, and in both cases the cephem and acrylate were recovered quantitatively.

As an alternative Michael acceptor, *p*-nitrobenzyl acrylate was prepared by esterification of acrylic acid with *p*-nitrobenzyl bromide in the presence of sodium carbonate. After work-up, the ester crystallised from the reaction mixture and purification of this crude product *via* Kugelrohr distillation afforded the pure ester in 15% yield. No attempt was made to optimise the yield of either of these esterification reactions. The fact that PNB acrylate was a solid posed an additional

problem in that these Michael reactions have previously been found¹⁶⁷ to occur in greatest yield when the Michael acceptor is present in vast excess as both reactant and solvent. Reaction of cephem (153) with *p*-nitrobenzyl acrylate in the presence of Triton B at 60°C (above the melting point of the acrylate) resulted in isomerisation of the cephem double bond. Additionally, two further components were observed on tlc which had identical R_f values to acrylic acid and *p*-nitrobenzyl alcohol and no incorporation of the acrylate into the cephem was observed.

An alternative entry to (267), using acrolein as the Michael acceptor, is outlined in Scheme 10. Initial formation of the C-4 aldehyde (268) followed by removal of the C-4 carboxyl group as outlined previously would result in the monosubstituted aldehyde (269) which could then be oxidised to the corresponding carboxylic acid (267).

However, when the Michael reaction between (153) and acrolein, in the presence of triethylamine, was carried out the reaction mixture rapidly became viscous and after several minutes had become completely solid. The solid was insoluble in most organic solvents and it did not prove possible to isolate any β -lactam containing material from the reaction mixture. Acrolein is known to polymerise in the presence of base¹⁶⁸ and in this case the catalysts for the Michael reaction appear to be sufficiently basic to induce polymerisation.

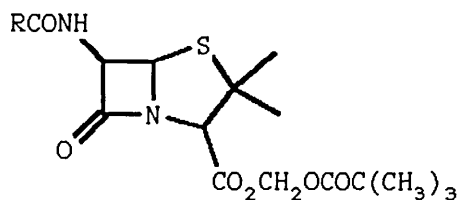


Scheme 10

In a final attempt to produce the desired carboxylic acid, hydrolysis of the methyl acrylate adduct (256) was examined. When (256) was stirred with dilute NaOH for 6 hours at room temperature, a single product was isolated in low yield. The infrared spectrum was found to have no β -lactam carbonyl stretch. Cleavage of the highly-strained β -lactam ring by dilute alkali is a well-known problem¹⁶⁹, and the hydrolysis products from this reaction were not further investigated. It was possible, however, that control of pH during the hydrolysis may have avoided problems associated with β -lactam cleavage.

Attempts to carry out the hydrolysis using a pH-stat apparatus* were hampered by the extreme insolubility of substrates (256) and (257) in most suitable organic solvents. During the course of the reaction, little consumption of the base was observed, and when pH 12 was reached, base addition was terminated. After working up the reaction mixture, starting material (256) was recovered in quantitative yield.

Recently it has been reported¹⁷⁰ that bis(tri-*n*-butyl) tin oxide (TBTO) can be used to de-esterify pivaloyloxymethyl penicillanates (270) and can also hydrolyse the methyl esters of several non β -lactam substrates (eg: methyl salicylate, methyl cinnamate and methyl hippurate). When methyl ester (256) was refluxed

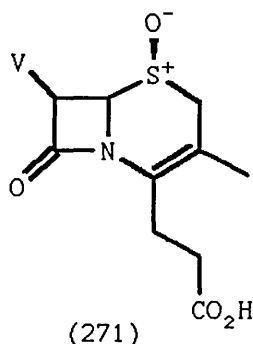


(270)

in toluene with excess TBTO, tlc after 30 minutes indicated conversion of starting material into a very polar component. Isolation of this compound gave a pale yellow solid (in low yield) which was found to contain a β -lactam carbonyl stretching frequency in the ir spectrum (1770cm^{-1}). In addition, a broad O-H stretch

A Metrohm Impulsomat E473 pH-stat apparatus was used for these experiments. The assistance of Dr John Harbridge is gratefully acknowledged.

at $2300-3280\text{cm}^{-1}$ indicated the presence of a carboxylic acid functionality. The mass spectrum showed a molecular ion at 392, corresponding to loss of the methyl ester from (256). The pmr spectrum also showed loss of the methyl ester though the C-4 ethyl protons were still present. A broad singlet ($\delta 12.24$) which exchanged with D_2O confirmed the presence of a carboxylic acid and so structure (271) is assigned. An



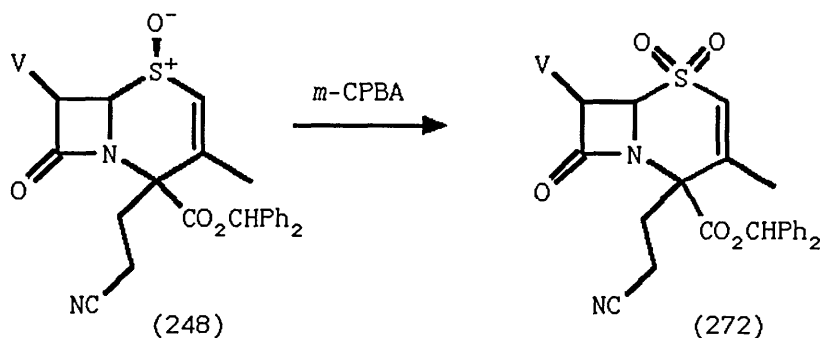
analytically pure sample could not be obtained, even after several recrystallisations.

2.1.7) Cephem Michael Adduct Sulphones.

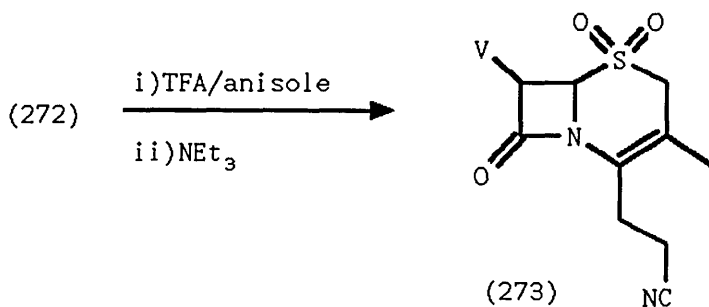
Recent interest²¹ in the use of cephem sulphones as inhibitors of an enzyme which has a degradative effect on lung tissue prompted investigation of Michael adduct sulphones.

Oxidation of acrylonitrile adduct sulfoxide (248) with excess *m*-CPBA at room temperature for 24h afforded a single product which had a similar pmr spectrum to sulfoxide (248). Mass spectroscopy indicated a molecular weight of 599 which was consistent with the incorporation of an oxygen atom into the starting material. Elemental analysis showed a molecular formula

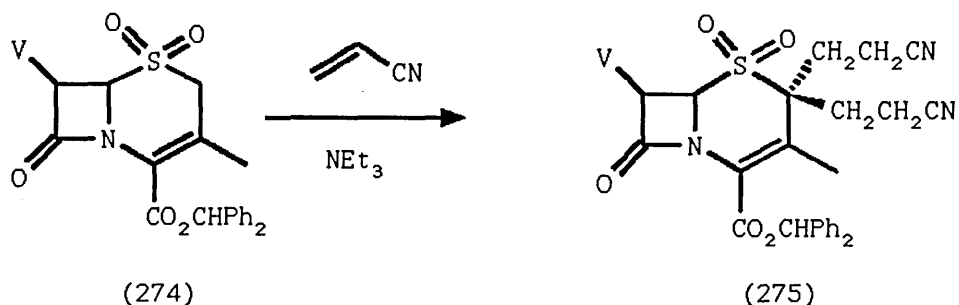
of $C_{32}H_{29}N_3O_7S$, and these data are consistent with sulphone (272). This sulphone was evaluated for elastase inhibitory activity, but none was observed.



C-4 Disubstituted ceph-2-em sulfoxides were shown in Section 2.1.4 to undergo de-esterification followed by decarboxylation to yield C-4 monosubstituted ceph-3-ems. When sulphone (272) was de-esterified with TFA/anisole followed by treatment of the crude acid with triethylamine overnight, a single non-polar product was formed. The infrared spectrum of the product showed the absence of any acid or ester groups which was also apparent from the pmr spectrum. The presence of two C-2 protons was indicated by an AB quartet ($\delta 3.45$ & 3.90 , $J=18\text{Hz}$). This data suggested that the sulphone acid had decarboxylated as in the sulfoxide series to afford (273). Elemental analysis was also consistent with this proposed structure. In an attempt to prepare (273) more



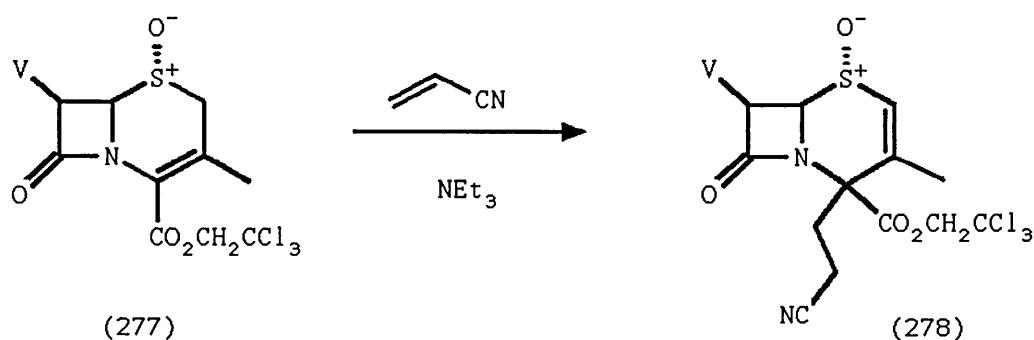
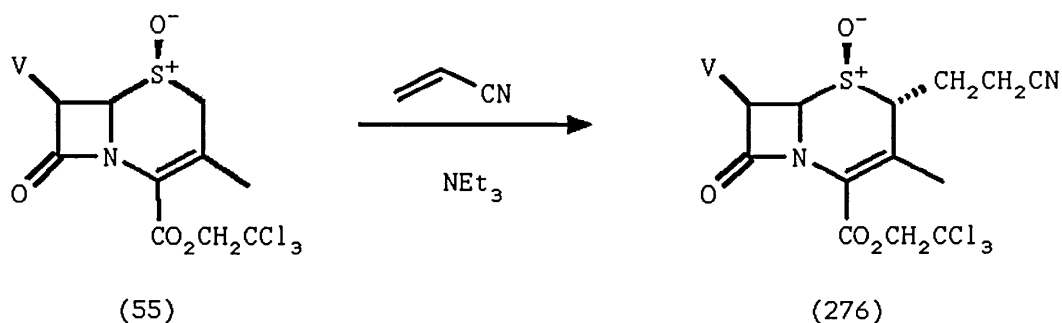
directly, sulphone (274) - prepared *via* oxidation of (153) with excess *m*-CPBA - was stirred with acrylonitrile in the presence of triethylamine. A single product was observed on tlc which was found to have a different R_f from (272). The infrared spectrum showed the presence of both nitrile and β -lactam carbonyl groups at 2250 and 1790 cm^{-1} respectively. In the pmr spectrum, no signals due to H-2 protons were present, either as a vinylic proton in a ceph-2-em or allylic proton(s) in a ceph-3-em. A multiplet attributed to the cyanoethyl group integrated for 8 protons indicating that two acrylonitrile residues had been incorporated into the product. An absorption maximum at 264nm in the UV spectrum indicated that the cephem double bond was still conjugated with the C-4 carbonyl. Hence a Δ -3 double bond is present and alkylation occurs twice at C-2 to give the bis(cyanoethyl) adduct (275). Mass spectroscopy



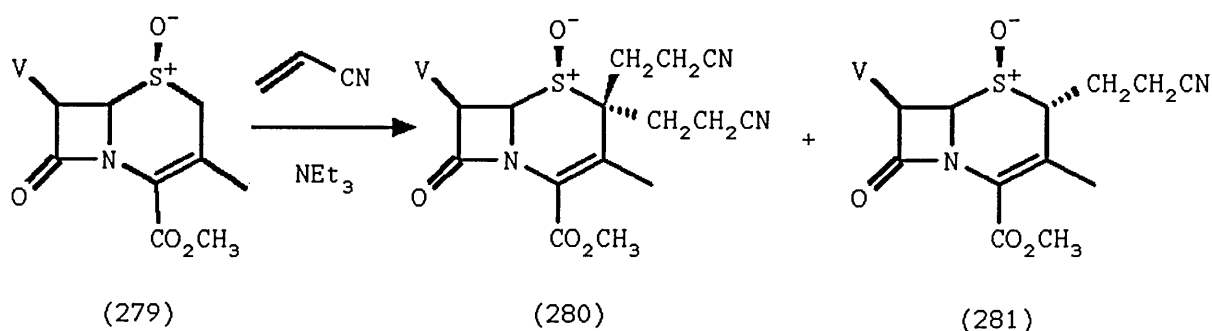
indicated a molecular ion at 652 and elemental analysis confirmed the molecular formula of (275) as $\text{C}_{35}\text{H}_{32}\text{N}_4\text{O}_7\text{S}$.

Regiospecificity in the Michael reaction of cephem sulfoxides has previously been reported¹⁷¹. Cephem β -sulphoxide (55) reacted with acrylonitrile in the

presence of triethylamine to give the 2-alkylated adduct (276), whereas the corresponding α -sulphoxide (277) afforded the 4-cyanoethyl adduct (278).



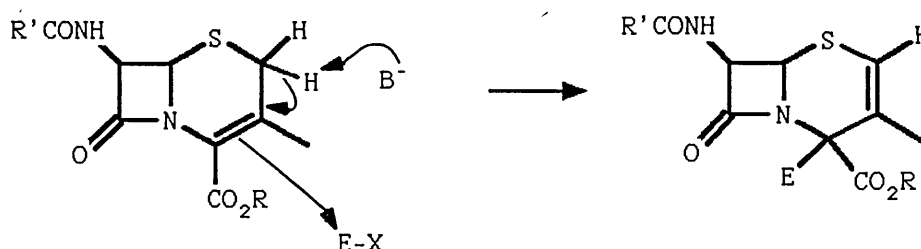
In the case of the methyl ester (279), reaction with acrylonitrile and triethylamine resulted in the 2,2-dialkylated ceph-3-em (280) as the major product and the 2-alkylated ceph-3-em (281) as the minor product¹¹⁴.



The regiospecificity of alkylation in cephems is further discussed in Section 2.2.4.

2.2) ALKYLATION OF CEPH-3-EMS AT C-4.

Since it had proved possible to convert C-4 disubstituted ceph-2-ems, obtained *via* Michael reactions into ceph-3-ems with a substituted ethyl group replacing the original C-4 carboxyl group, the scope of this scheme was examined. In particular, the use of stronger bases with a variety of electrophiles was viewed as a logical extension to the Michael reactions.

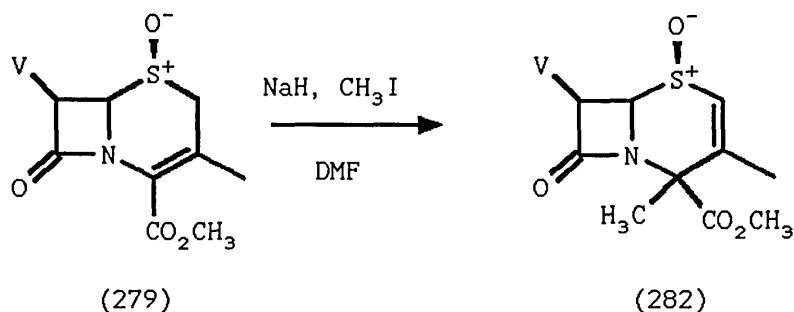


A variety of bases could be employed for this reaction, the only stipulation being that they should not attack the β -lactam ring. Sodium hydride^{113,159,162,172,173} and lithium diisopropylamide^{54,55,174,175} have both been reported as being effective in the deprotonation of the C-2 position in cephems, and these bases were selected initially in an investigation of C-4 alkylation.

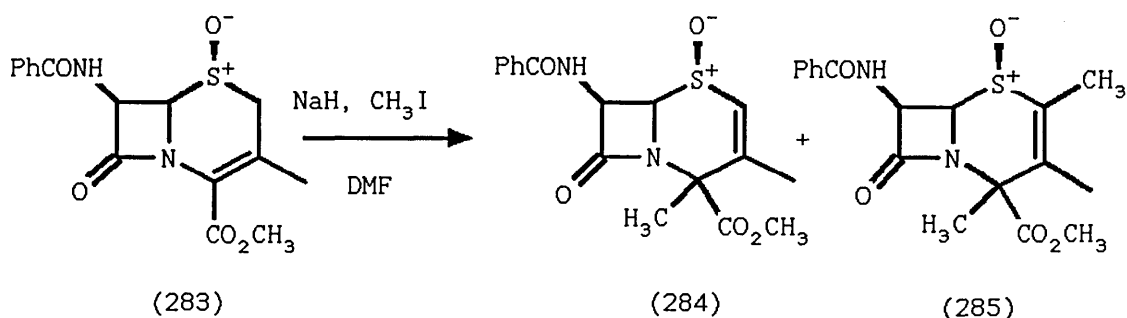
2.2.1) Reaction with Sodium Hydride.

The use of NaH as a base in cephalosporin alkylation reactions was first reported by Dolfini in 1972¹⁷². The reaction of ceph-3-em β -sulphoxide (279) with sodium hydride in the presence of an electrophile such as iodomethane was claimed to produce the 4-methylceph-2-em

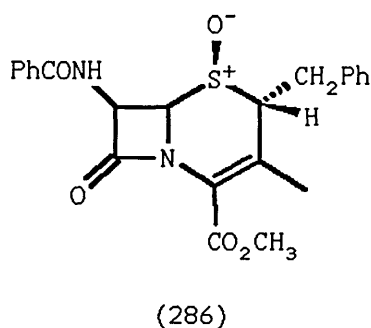
(282) exclusively. Subsequently, however, Sankyo



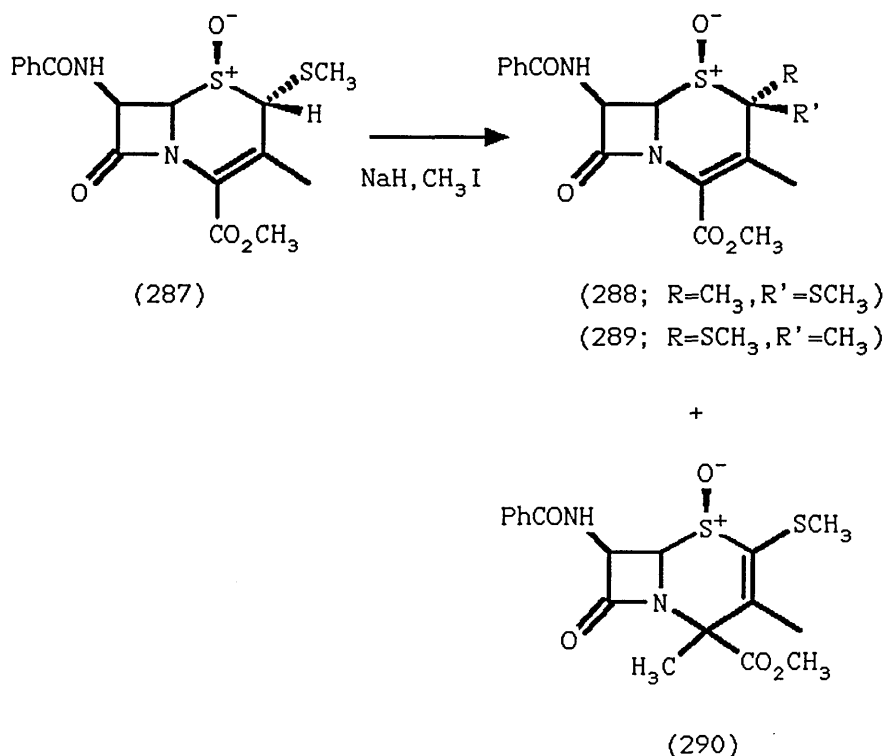
workers¹⁶² repeated this reaction with cephem sulphoxide (283) under the same conditions and obtained, as the major product, 4-methylceph-2-em (284) along with a 10% yield of the 2,4-dimethylated cephem (285). When benzyl



bromide was used as the electrophile, the 2-benzyl ceph-3-em (286) was isolated along with the benzyl analogues of (284) and (285). The same authors¹⁵⁹ also

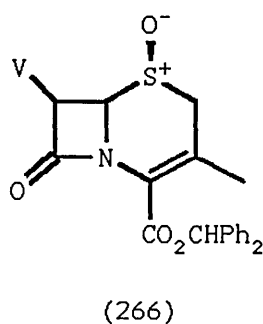


utilised sodium hydride as base in the alkylation of 2 α -thiomethylcephem (287) (prepared *via* methylthiolation of (283) with LDA and MsSCH₃) to give a mixture of both 2- and 4-methylated products (288-290). Sodium hydride



has also been cited (without examples) in patents detailing C-4 alkylations in cepheids^{113, 173}.

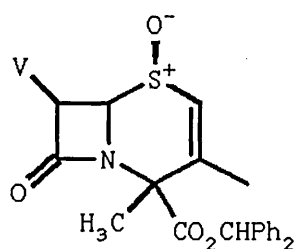
When sulfoxide (266) was stirred in DMF containing iodomethane with 1.2 equivalents of sodium hydride, a two component mixture was observed after 1.5 hours. The



mixture was easily separated by column chromatography and the major, more polar component was found to have a UV maximum at 253nm. Ceph-3-em systems have been found¹⁷⁶ to exhibit an ultraviolet absorption maximum at ca. 260nm, and a range of cep-3-em absorption maxima

have been tabulated¹⁷⁷. All were found to be in the range of 260-268nm when a C-4 carboxyl group was present. When the UV absorption of ceph-2-em esters was examined¹⁵⁷, maximum absorption was found between 245-250nm, primarily as a consequence of the loss of conjugation between the Δ -3 double bond and the C-4 ester function.

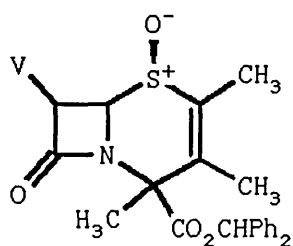
Thus, in the reaction of (266), the hypsochromic shift observed in the UV spectrum indicates the presence of a ceph-2-em system derived from alkylation at C-4. The ceph-2-em structure was confirmed by the pmr spectrum where a downfield doublet (δ 6.73, J =1.2Hz) is observed due to the C-2 vinyl proton which is coupled to the 3-methyl group. An additional 3 proton singlet showed the incorporation of a single methyl group at C-4. The structure of the major component from the alkylation is therefore assigned as (291), and elemental analysis and mass spectroscopy data are in accord with this structure.



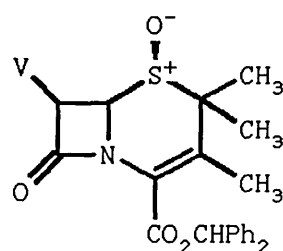
(291)

In the case of the minor component, a molecular mass of 558 is observed, suggesting that 2 methyl groups had been added to (266). The pmr spectrum shows that three

methyl groups are present, two of which couple with each other ($\delta 1.64$ and $\delta 2.20$, $J=0.9\text{Hz}$). The third methyl group ($\delta 2.08$) is a singlet. No signals due to C-2 protons are observed. Therefore, reaction with 2 equivalents of iodomethane has occurred resulting in either the 2,3,4-trimethyl ceph-2-em (292) or the 2,2,3-trimethylceph-3-em (293). The presence of



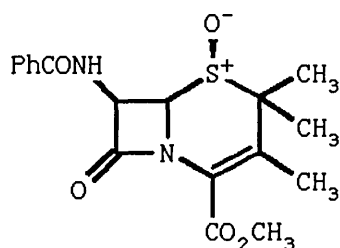
(292)



(293)

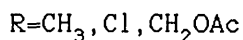
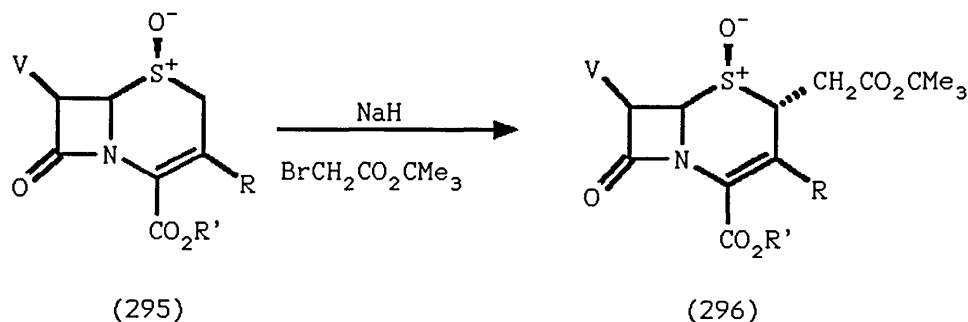
coupling between two of the methyl groups was originally attributed to vinylic coupling between C-3 methyl and the C-2 methyl group in (292), however an absorption maximum at 264nm in the ultraviolet spectrum indicates a ceph-3-em double bond. This suggests that the minor product is the 2-disubstituted ceph-3-em (293).

Previously, Oida *et al*¹⁶² noted the 'doublet-like' character of the C-3 and C-2 methyl groups in (285), however no confirmation of the position of the double bond in this molecule was given. On the basis of the results detailed above it is possible that the minor product obtained by these workers is not (285) but the 2,2-dimethylated isomer (294).

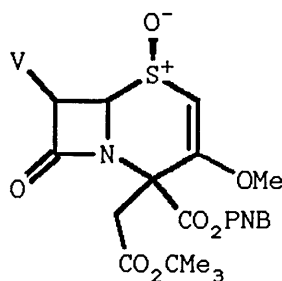


(294)

Oxidation of cephem s to the β -sulphoxide is known to activate the C-2 position^{54, 114}, allowing the C-2 protons to be readily removed by base and the resultant C-2 carbanion to be delocalised into the sulphoxide moiety. It is not surprising therefore that some C-2 alkylation should occur when cephem sulphoxides are reacted with NaH and CH_3I , however with other electrophiles, exclusive C-2 alkylation has been reported⁵⁵. When sulphoxide (295) was deprotonated with sodium hydride followed by alkylation with *t*-butyl bromoacetate, (296) was obtained exclusively. Only when

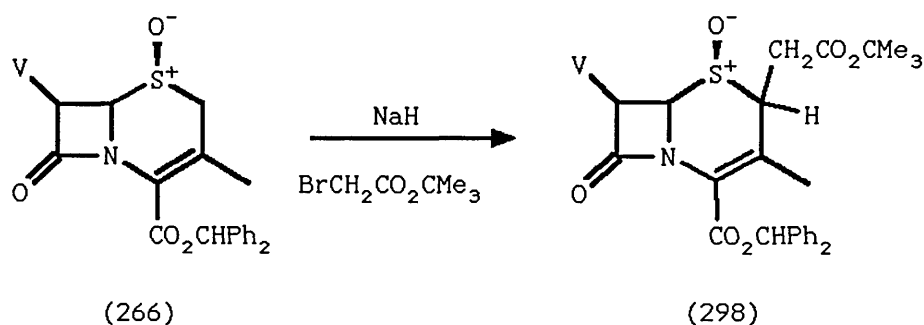


(295; $\text{R} = \text{OMe}, \text{R}' = \text{PNB}$) was used did any alkylation occur at C-4, with ceph-2-em (297) being obtained in 30% yield in addition to (296; $\text{R} = \text{OMe}, \text{R}' = \text{PNB}$) (43%).



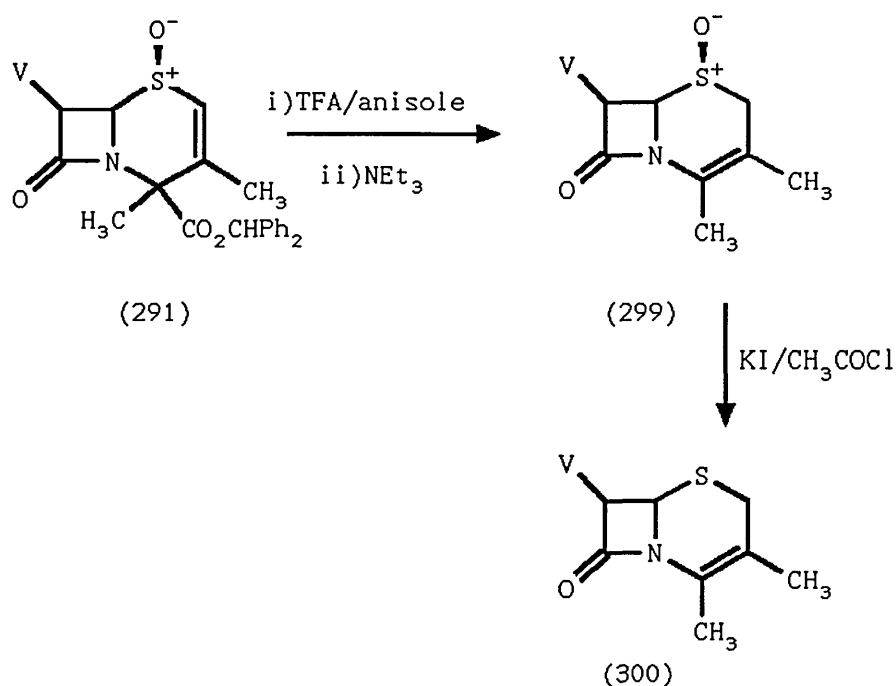
(297)

Since ceph-3-em β -sulfoxides have been shown above to react with sodium hydride and iodomethane to give predominantly C-4 alkylated products, the formation of C-2 alkylated products of structure (296) appears somewhat anomalous. In order to establish whether any difference in regiospecificity occurred with *t*-butyl bromoacetate, sulfoxide (266) was stirred with the alkylating agent followed by addition of NaH. A single product was obtained as a white powder (64%) and the presence of UV absorption at 268nm confirmed the presence of a ceph-3-em system. The mass spectrum indicated a molecular mass of 644 corresponding to the incorporation of a *t*-butyl acetate residue. The pmr spectrum also showed the presence of a single *t*-butyl acetate group with a 9 proton singlet due to the *t*-butyl ester and a double doublet for each of the methylene protons of the acetate group. Each of these protons is split by the remaining C-2 proton which consequently is observed as a double doublet. The chemical shift for this C-2 proton was considerably more upfield (δ 4.00) than that observed for the C-2 proton in ceph-2-ems, again confirming the ceph-3-em structure of the product.

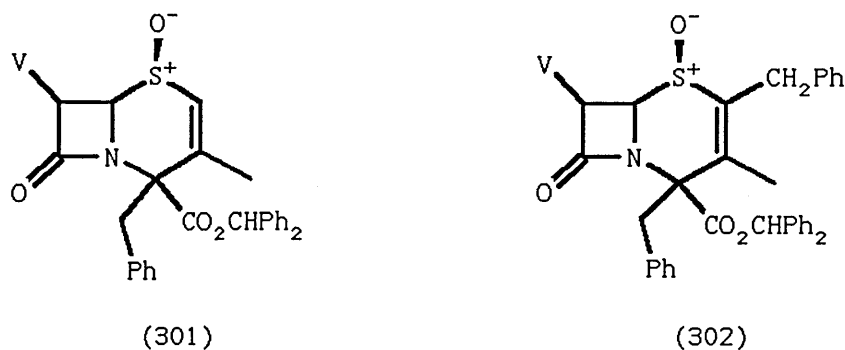


The regiospecificity of alkylation in cephem sulfoxides is extensively discussed in Section 2.2.4 and consequently no attempt will be made to rationalise these results here.

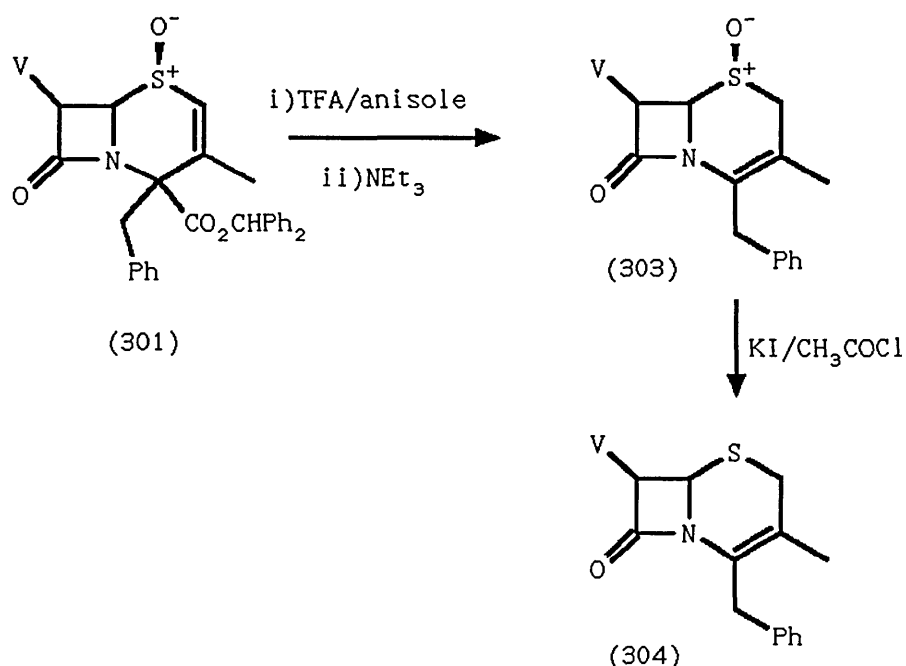
In order to extend the scope of the de-esterification/decarboxylation route to C-4 monosubstituted ceph-3-ems detailed in Section 2.1.4, the C-4 methyl derivative **(291)** was stirred with TFA/anisole. Decarboxylation of the resultant acid *in situ* with triethylamine gave the 3,4-dimethylceph-3-em **(299)** in 76% yield. A UV maximum at 251nm confirmed the absence of a C-4 carboxyl group conjugated to the ceph-3-em system. The pmr spectrum contained an AB quartet ($J=18.2\text{Hz}$) for the two C-2 protons as normally observed for a ceph-3-em and elemental analysis confirmed the formula as $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_4\text{S}$. De-oxygenation of the sulfoxide proceeded readily with $\text{KI}/\text{CH}_3\text{COCl}$ ⁴³ to give sulphide **(300)** in a 30% overall yield from **(266)**.



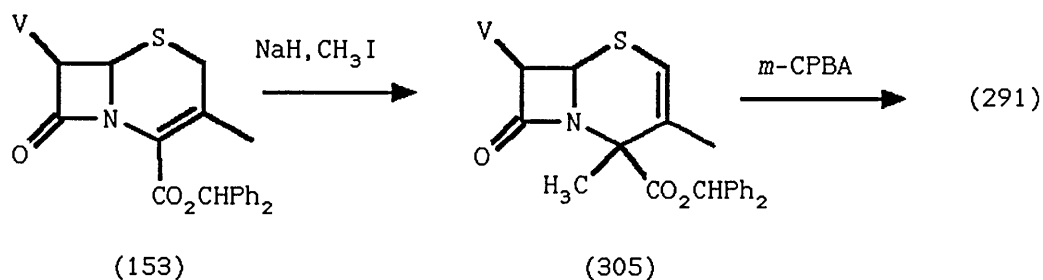
When benzyl bromide was used as the electrophile, the 4-benzyl and 2,4-dibenzyl adducts **(301)** and **(302)** were obtained in 40% and 20% yields respectively. This result is in accordance with that previously observed¹⁶², and all spectroscopic and analytical data were in agreement with the structures assigned.



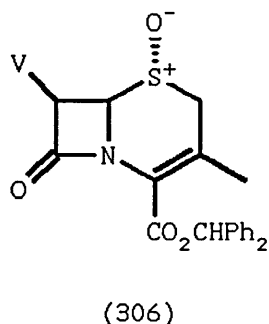
De-esterification of **(301)** with TFA/anisole followed by decarboxylation with NEt_3 *in situ* gave the 4-benzylceph-3-em **(303)** which de-oxygenated when treated with $\text{KI/CH}_3\text{COCl}$ to give **(304)** in an overall yield of 10% from **(266)**.



Since it was believed that the primary reason for competition between C-2 and C-4 alkylation was due to activation of the 2 position by the β -sulphoxide, reaction of the cephem sulphide under identical conditions should give predominantly the C-4 alkylated product. When sulphide (153) was stirred with NaH and iodomethane, a single component was observed on tlc after 1 hour. The pmr spectrum of the isolated product (62%) showed the incorporation of only one methyl group, and the presence of a doublet ($\delta 6.46$, $J=1.2\text{Hz}$), which was coupled with the C-3 methyl group, indicated a ceph-2-em structure which was confirmed by a UV absorption maximum at 258nm. Thus, alkylation had occurred at C-4 and oxidation of this product (305) gave a sulphoxide which was identical (tlc, mp, pmr) to the previously prepared sulphoxide (291).



The regiospecificity of these alkylation reactions was similar to that previously observed during Michael reactions¹¹⁴. In an attempt to complete the study of regiospecificity under sodium hydride induced alkylation, α -sulphoxide (306) was prepared by reaction

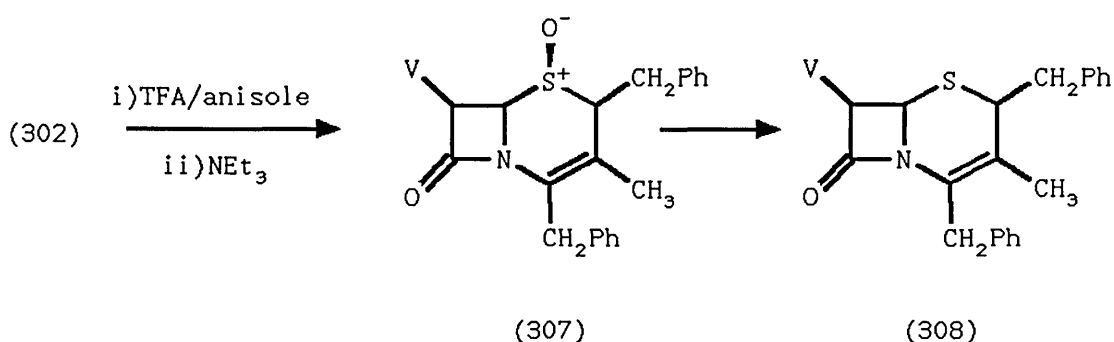


of the corresponding sulphide (153) with N-monochlorourethane in wet THF³⁴. Reaction of (306) with sodium hydride in the presence of iodomethane gave rise to a complex mixture from which no pure material could be isolated. Jaszberenyi has previously noted⁵⁰ that cephem α -sulphoxides fail to participate in the Mannich reaction. This is attributed to steric factors due to the α -sulphoxide which prevent attack on the C-2 position, and a similar situation pertains in the reaction of (306) with sodium hydride and iodomethane.

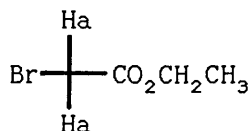
In all the cases detailed above the 4-substituent added to the cephem is believed to adopt 4 β -

stereochemistry. This is a consequence of repulsion between the N-5 lone pair (on the α -face) and the C-4 carbanion which must form on the β -face (see Section 2.1.2).

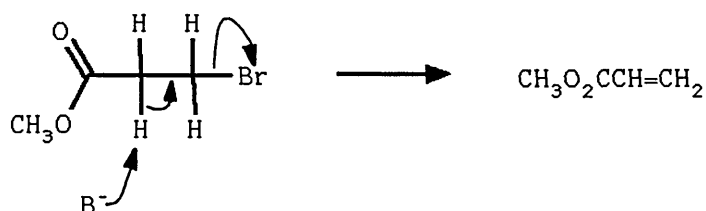
The low yields of the 2,4-dibenzylcephem (**302**) obtained from the reaction precluded any attempt to de-esterify and decarboxylate the C-4 ester group to produce (**307**) which could subsequently be de-oxygenated to afford sulphide (**308**).



In order to extend the scope of C-4 alkylation reactions to substituents other than simple alkyl or aryl functions other electrophiles were studied. When sulphide (**153**) was stirred with sodium hydride in the presence of ethyl bromoacetate in DMF, after 10 hours no reaction was observed by tlc. This lack of reactivity can be explained by the very high acidity of the α protons, Ha, in the electrophile. Thus, the cephem carbanion abstracts Ha resulting in recovered starting material, rather than undergoing a nucleophilic substitution reaction. To try and decrease the acidity



of these protons, consequently making alkylation more favourable than reprotonation, methyl 3-bromopropionate (309) was used as the electrophile. Again no reaction was observed though a strong smell of methyl acrylate was present in the reaction mixture. It is believed that elimination of HBr from (309) occurs under the reaction conditions employed. Ethyl chloroformate,



(309)

which has no α protons, also failed to react under these conditions.

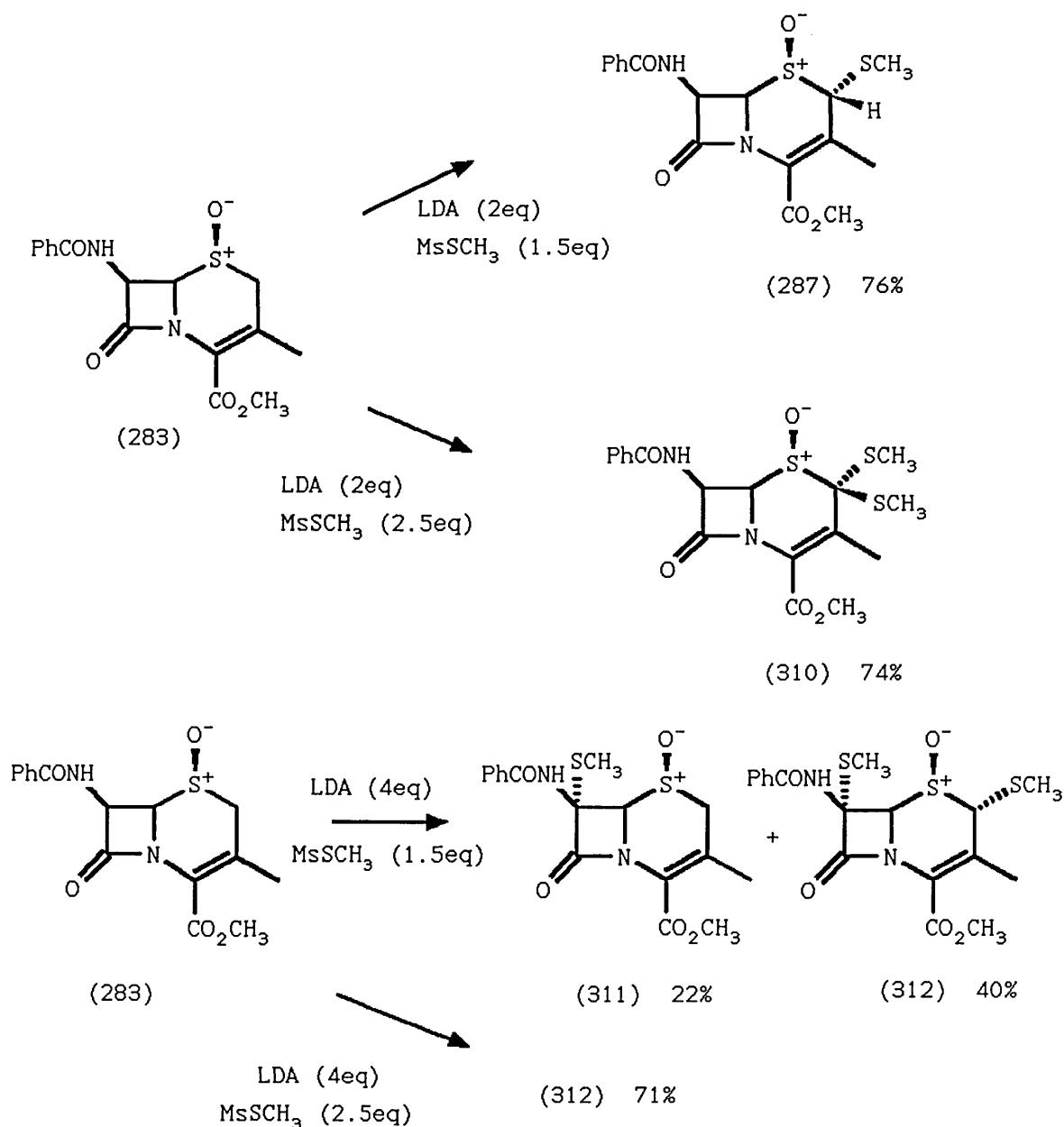
t-Butyl bromoacetate has previously been found⁵⁵ to react at C-2 in cephem sulfoxides and the failure of ethyl bromoacetate to react under identical conditions can be rationalised by the enhanced positive inductive effect of the *t*-butyl group compared to the ethyl group. This will consequently decrease the acidity of the α protons in the *t*-butyl acetate, allowing substitution to become more favourable than protonation.

2.2.2) Reaction with Organolithium Bases.

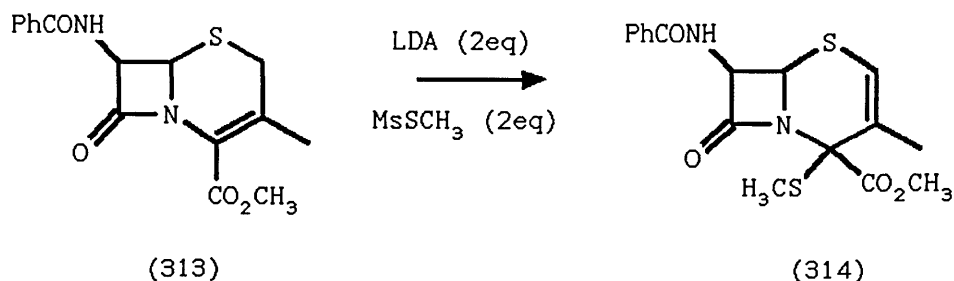
LCIA and LDA have previously been utilised for methylthiolation^{159,174} and alkylation^{54,162,175} of the cephem nucleus. Due to the very high basicity of LDA, the possibility exists for removal of the NH, C-2 and C-7 protons and consequently alkylation is very

sensitive to the ratio of base and electrophile used.

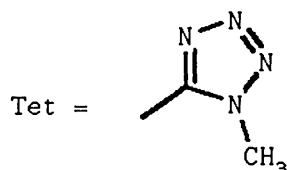
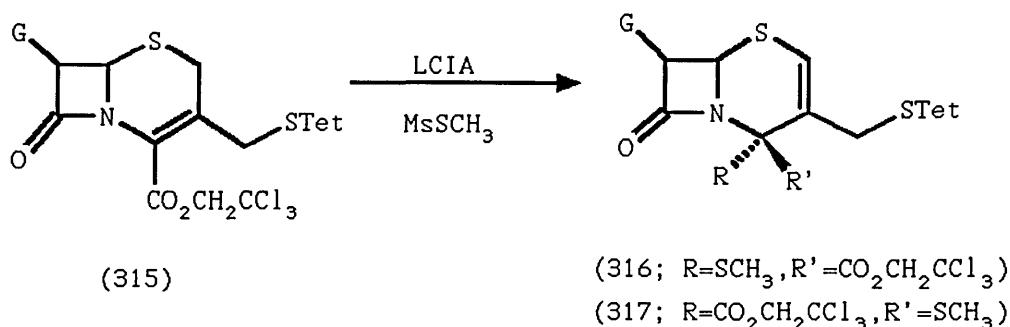
Thus, in the methylthiolation of sulfoxide (283)¹⁵⁹, 2 equivalents of base and 1.5 equivalents of MsSCH₃ give rise to the 2 α -thiomethylcephem (287). Increasing the concentration of electrophile to 2.5 mole equivalents resulted in formation of solely the 2,2-bis(thiomethyl)cephem (310). Similarly, increasing the concentration of base affects the site of methylthiolation.



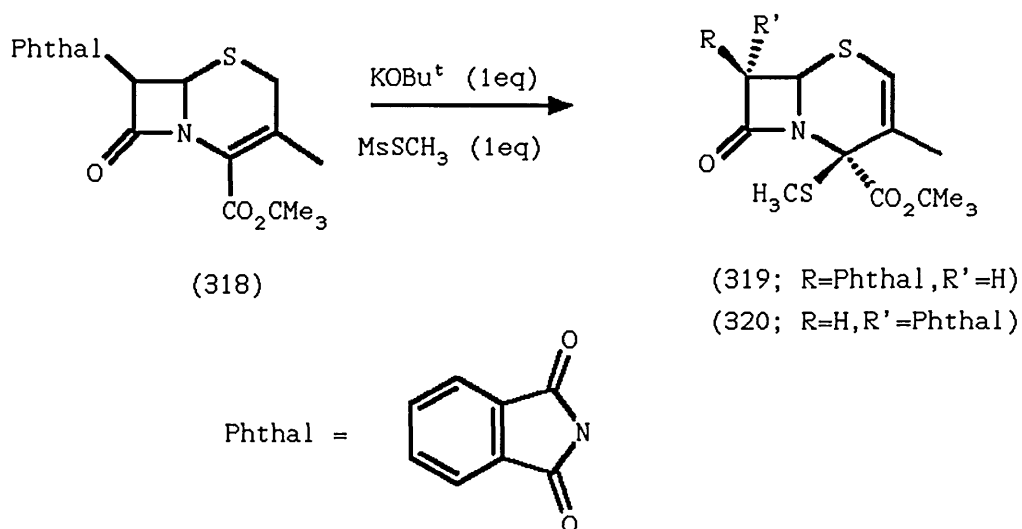
The enhanced stability of the C-2 carbanion due to the β -sulphoxide group is not present in the corresponding sulphide series and so methylthiolation occurs at C-4 to afford (314). In a further investigation of the



methylthiolation of cephem sulphoxides¹⁷⁴, Squibb workers found that potassium *t*-butoxide, followed by MsSCH_3 , also gives the 2 α -thiomethyl and 2,2-bis-(thiomethyl)cephem sulphoxides. In the sulphide series, reaction of (315) with LCIA and MsSCH_3 (2 equivalents of each) afforded a mixture of the epimeric 4-thiomethyl ceph-2-ems (316) and (317). The 7-phthalimidocephem

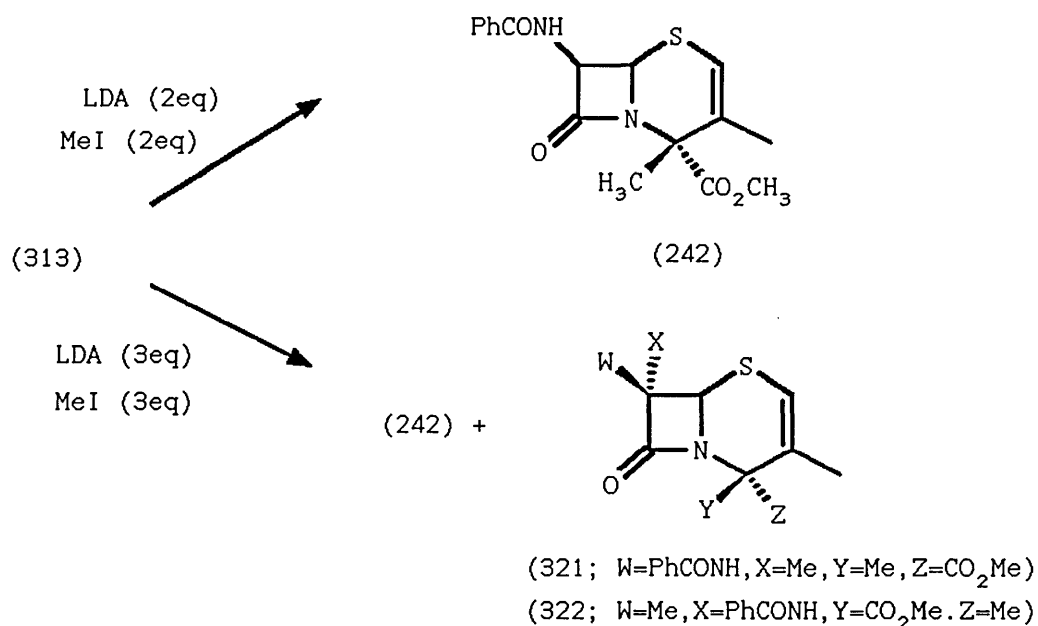


(318) also reacted regiospecifically at C-4 with potassium *t*-butoxide and methyl methanethiosulphonate to give (319) and (320). The latter compound results from epimerisation at C-7.

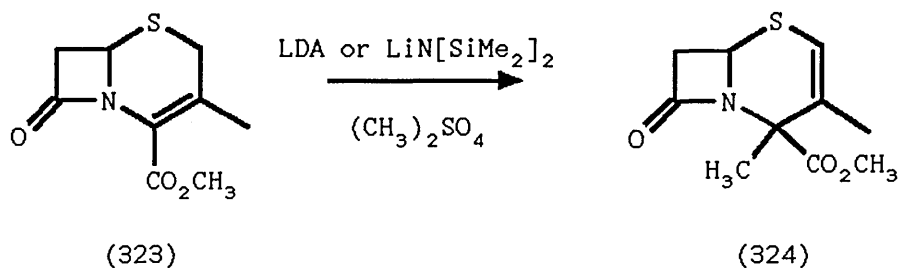


Similar results to those obtained with methylthiolation reactions were observed when iodomethane was employed as electrophile¹⁶² in the alkylation of sulphide (313).

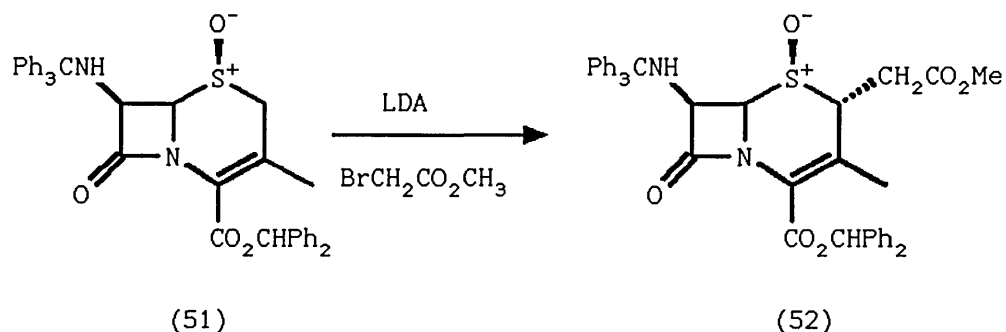
The use of 2 equivalents of each of LDA and CH₃I resulted in the 4β-methylceph-2-em (242), whereas 3 equivalents of both base and electrophile gave (242) and the 4,7-dimethylated ceph-2-ems (321) and (322).



Several electrophiles have been added¹⁷⁵ to the cephem analogue (323) via deprotonation with LDA or lithium hexamethyldisilazide, followed by treatment with dimethyl sulphate, methyl chloroformate, *p*-methoxybenzyl bromide or benzyl chloroformate. In all cases only the 4-substituted ceph-2-em, such as (324), was obtained.



When the cephem sulfoxide (51) was reacted⁵⁴ with LDA and methyl bromoacetate (1 mole equivalent of each), the 2 α -substituted ceph-3-em (52) was obtained.



To briefly summarise the results previously obtained : 2 equivalents of LDA are normally required to produce alkylation in cephems, however non-amide side chains such as phthalimido or tritylamino require only 1 equivalent. This suggests that with 7 β -amide side chains, the first equivalent of base removes the NH proton (the acidity and ease of removal of which is decreased for the tritylamino side chain), and the second equivalent of base removes a C-2 proton. In

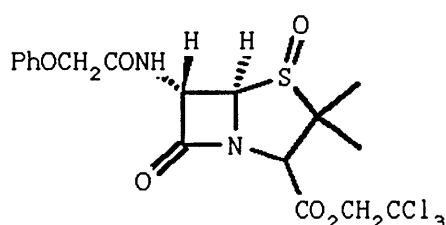
cephem sulfoxides, alkylation occurs at C-2 whereas sulphides react exclusively at C-4. In both cases, when further base is added, alkylation can occur at C-7. The regiospecificity of alkylation in ceph-3-em is further discussed in Section 2.2.4 after the results obtained as part of this project are presented.

2.2.3) Reaction of Ceph-3-em Sulphides with LDA.

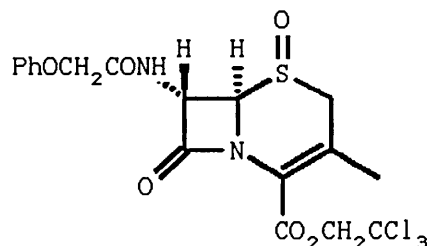
After several attempts to react sulphide (153) with LDA and iodomethane, a procedure was devised whereby alkylation occurs in reasonable yield. The cephem is deprotonated with preformed LDA (2 mole equivalents) at -70°C followed by addition of electrophile (2 mole equivalents) and stirring at -70°C for 1-2 hours. The reaction mixture is then allowed to warm to 0°C before quenching with saturated aqueous NH_4Cl solution.

When ceph-3-em (153) was reacted with iodomethane under these conditions, tlc indicated conversion into a single, less polar component along with some baseline material. Chromatography afforded a single β -lactam containing product but the pmr spectrum clearly indicated the presence of two components. All peaks in the spectrum were duplicated, but it was still possible to discern an additional singlet integrating for 3 protons in each component. Furthermore, the signal assigned to the C-3 methyl group was split ($J=1.2\text{Hz}$) by coupling with the single C-2 proton. Comparison of the integrals for the two components indicated a ratio of approximately 3:1. The major difference between the two

spectra was observed in the signals attributed to the C-6 and C-7 protons of the minor component which had a coupling constant of 1.6Hz as opposed to the more usual 4.6Hz observed in the major product. This decrease in coupling constant has previously been observed^{178,179} in 6-epipenicillins (325) and 7-epicephalosporins (326).

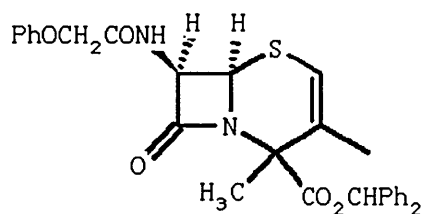


(325)

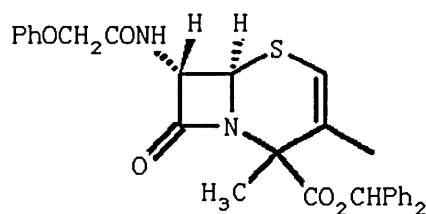


(326)

Epimerisation at C-7 in cephalosporins gives rise to an upfield shift in the position of the H-7 signal and such a shift of 0.58 was observed in the minor component of this reaction. It was therefore apparent that the 2 components were the *cis*- and *trans*- β -lactams (305) and (327). Attempts to separate the components in order to



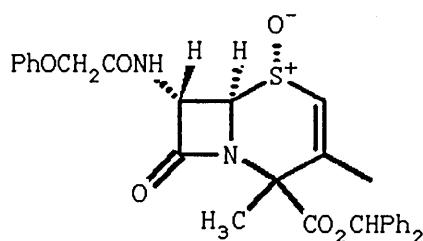
(305)



(327)

confirm this hypothesis failed due to their very similar R_f values. It was reasoned that oxidation of the reaction mixture with *m*-CPBA would give rise to the β -sulphoxide in the case of (305) whereas oxidation of (327) would result in the α -sulphoxide. Oxidation of the reaction mixture did indeed afford a two-component

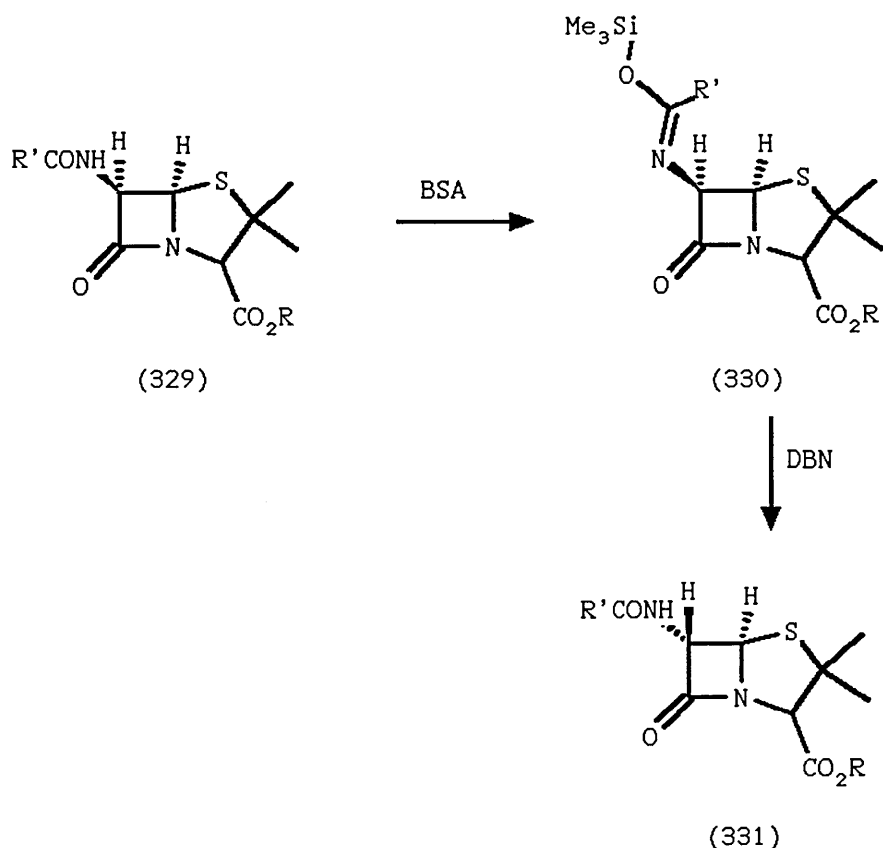
mixture which was readily separated by column chromatography. The major component was found to be identical (tlc,mp,pmr) to an authentic sample of the 4-methylceph-2-em sulphoxide (291) prepared by reaction of the ceph-3-em sulphoxide with sodium hydride and iodomethane. The more polar, minor component also had a molecular mass of 544 and elemental analysis confirmed the formula as $C_{30}H_{28}N_2O_6S$, reinforcing the hypothesis that the two components were epimers. A coupling constant of 1.7Hz between H-6 and H-7 confirmed that epimerisation had occurred, and the presence of a single vinylic C-2 proton indicated a ceph-2-em and hence C-4 alkylation. On the basis of these data, structure (328) is assigned. Epimerisation of cephalosporins has not



(328)

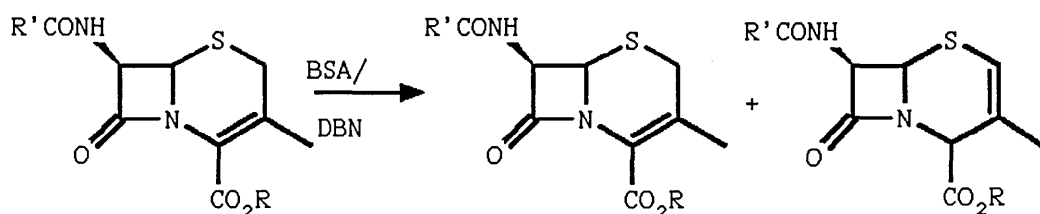
been studied to the same extent as in the penicillin series. Initial attempts to convert 6β -amidopenicillins into their 6α -epimers were limited to side chains which were not secondary amides, such as diacylamino- or acylalkylamino¹⁸⁰⁻¹⁸². The first proton to be removed by base is the NH proton, and further deprotonation at C-6 is prevented due to the proximity of the two negative charges¹⁸². Protection of the NH proton in secondary amide side chains as the silyl iminoether

(330) (using BSA), followed by reaction with a base allows removal of the C-6 proton to give the 6-epimer (331)¹⁸³. A direct method of epimerisation of

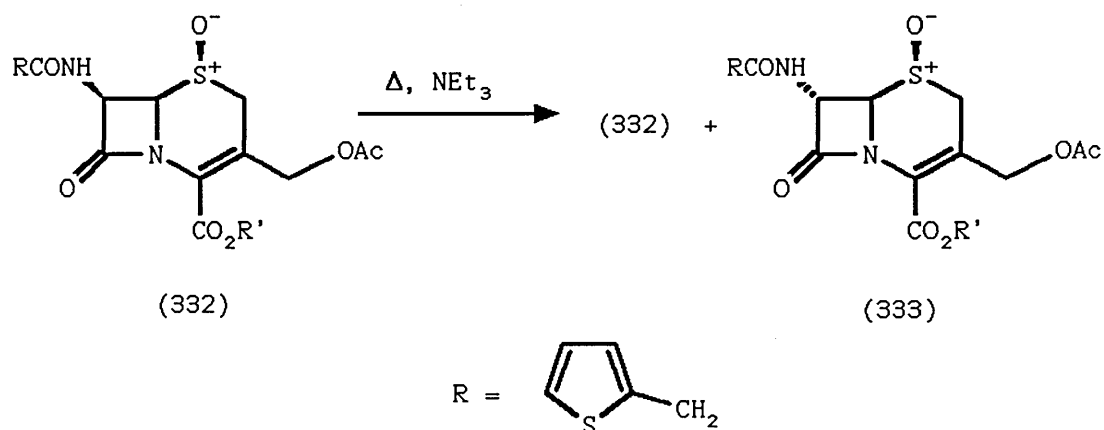


penicillins bearing a secondary amide side chain has also been reported¹⁸⁴. Reaction of (329) with 2.5 equivalents of LDA followed by quenching resulted in a mixture of recovered starting material and its 6-epimer (331) in a ratio of 1:4.

In the cephalosporin series, BSA/DBN did not produce epimerisation as expected, however isomerisation of the ceph-3-em double bond was observed¹⁷⁹. The

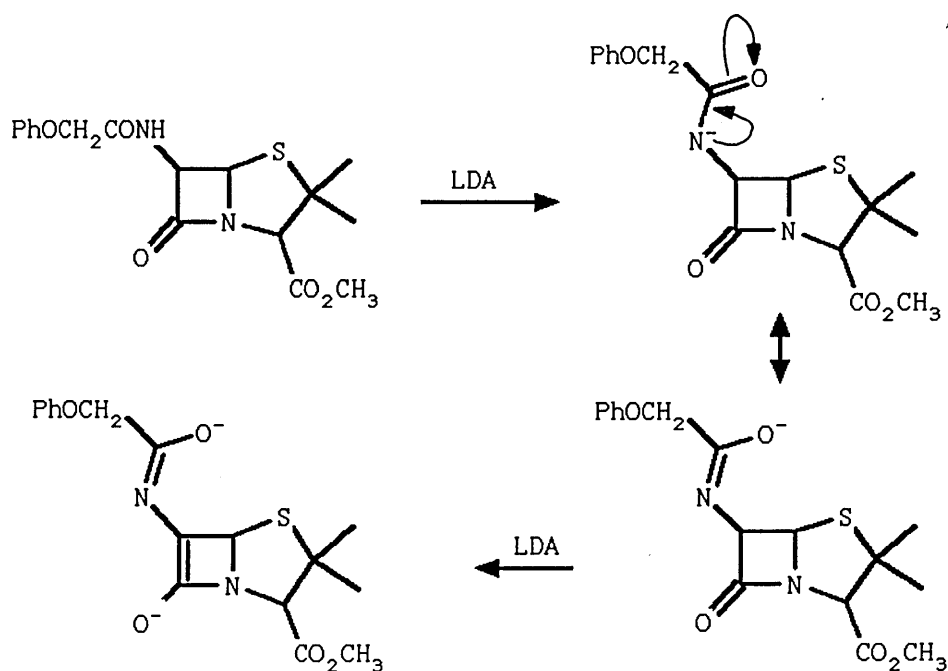


epimerisation of cephem sulfoxides (332) by heating in the presence of triethylamine results in a 1:1 mixture of the epimer (333) and recovered starting material^{179,185}. In this case, the Δ -3 double bond is



thermodynamically more favourable than the Δ -2 isomer⁴³, hence base can abstract the C-7 proton as in the penicillin series to give the 7-epicephalosporin.

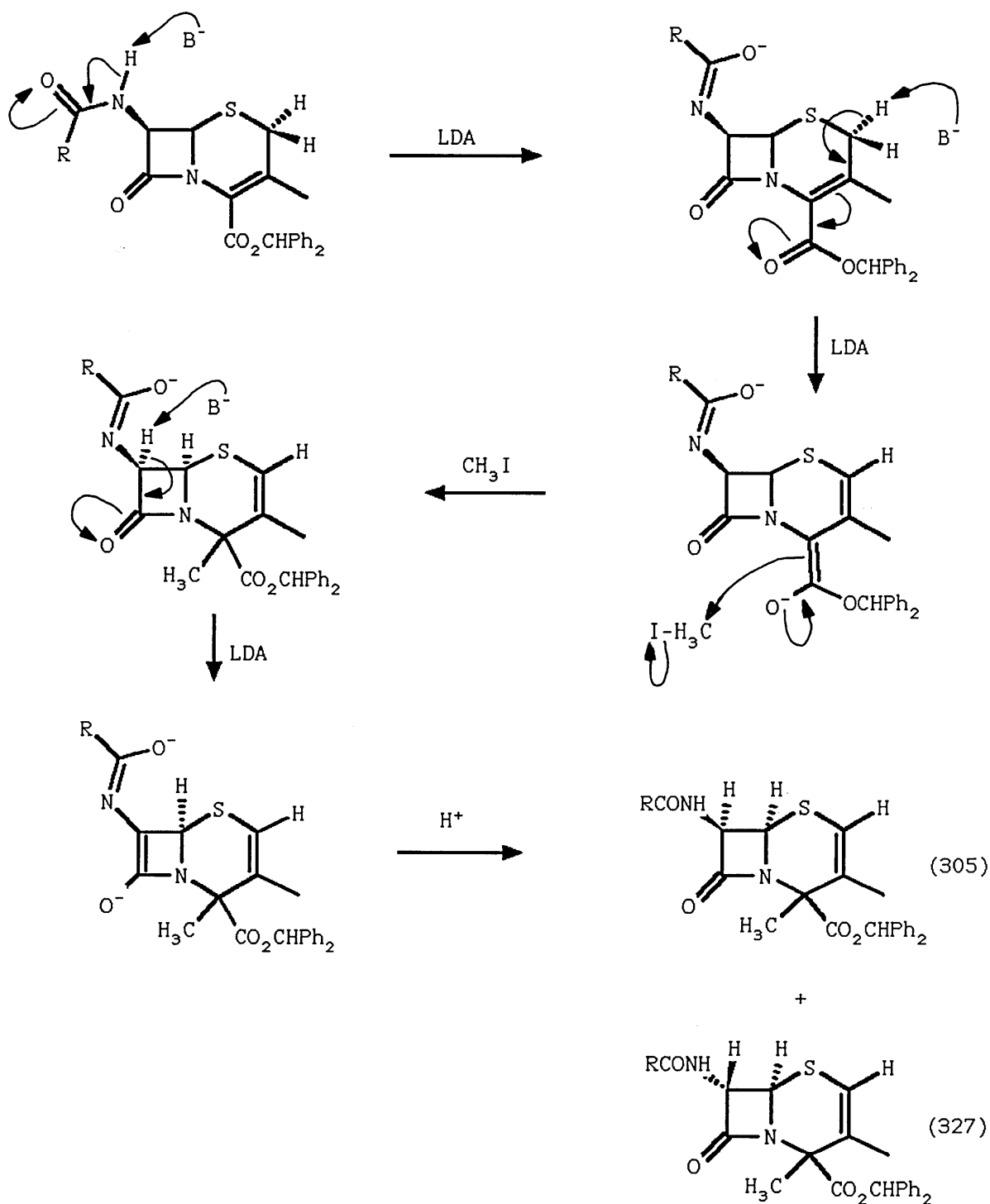
By further considering the work of Koppel¹⁸⁴ in the LDA induced epimerisation of penicillins, the formation of epimers (305) and (327) can be rationalised. The first mole of base removes the amide side-chain proton, and a second mole of base removes the C-6 proton to give dianion (334).



(334)

In an analogous manner, when cephem (153) is reacted with LDA, the NH proton is the most acidic and hence will be removed first. A second equivalent of base is then required to deprotonate the cephem nucleus, however, unlike the penicillins either the C-7 or a C-2 proton can be removed by this second mole of base. Since both products obtained from the reaction of (153) are C-4 alkylated (resulting from C-2 deprotonation), and the minor product is the 7-epimer (resulting from C-7 deprotonation) it is proposed that the second equivalent of base removes the C-2 proton rather than the C-7 proton. This is in accordance with previous findings^{159, 162} where C-7 alkylation was only observed when more than 2 equivalents of base were employed. In the alkylation of (153) it is believed that slightly more than 2 equivalents of LDA were used, and in the absence of excess electrophile, the C-7 carbanion is

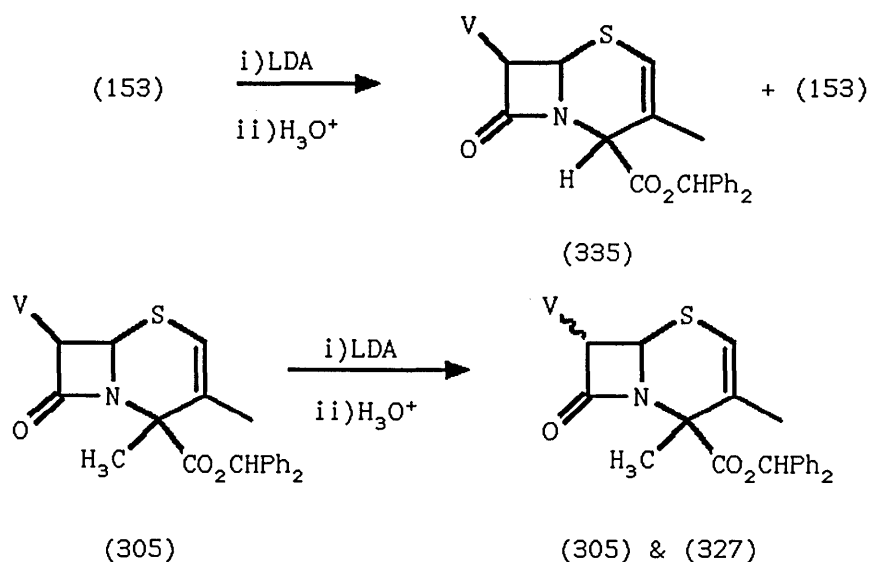
re-protonated on quenching to give a mixture of epimers (305) and (327). The reaction sequence is detailed in Scheme 11.



Scheme 11

If the mechanism proposed above is correct, it should follow that deprotonation of cephem (153) with exactly

two equivalents of LDA followed by direct quenching (ie: no electrophile added) should result in a mixture of recovered starting material and the Δ -2 isomer (335). Similarly, a C-4 disubstituted ceph-2-em such as (305) should react with 2 equivalents of LDA to give an epimeric mixture of (305) and (327). Thus, sulphide



(153) was reacted with 2 equivalents of LDA (prepared from freshly standardised butyl lithium¹⁸⁶) under identical conditions to those employed in the alkylation (without the addition of electrophile), and quenched with NH₄Cl. TLC indicated a 2-component system which was similar to a mixture of ceph-2-em (335) and (153) prepared *via* NEt₃ isomerisation of (153). Oxidation of the reaction mixture with *m*-CPBA gave a single product whose pmr spectrum was identical with that of sulfoxide (266). No epimerisation was observed and this was confirmed by H-6/H-7 coupling of 4.6Hz indicative of a *cis*- β -lactam. Long range coupling between H-6 and the

more upfield doublet of the C-2 AB quartet (previously attributed to the H-2 α proton¹⁶⁴) was also observed (1.2Hz). Such coupling is only present in the β -sulphoxide¹⁶⁴, thus proving that the products from this reaction both have a 7 β -amide side chain, and hence epimerisation has not occurred.

When ceph-2-em (305) was reacted with 2 equivalents of LDA and quenched directly without adding an electrophile, the reaction mixture initially appeared to contain only 1 compound. After oxidation with *m*-CPBA however, 2 components with similar R_f values to *cis*- and *trans*- β -lactams (291) and (328) were isolated, after chromatography, in a 2:1 ratio. Spectroscopic analysis confirmed that the products were identical to (291) and (328), with a decreased coupling constant between H-6 and H-7 of 1.7Hz in the minor component.

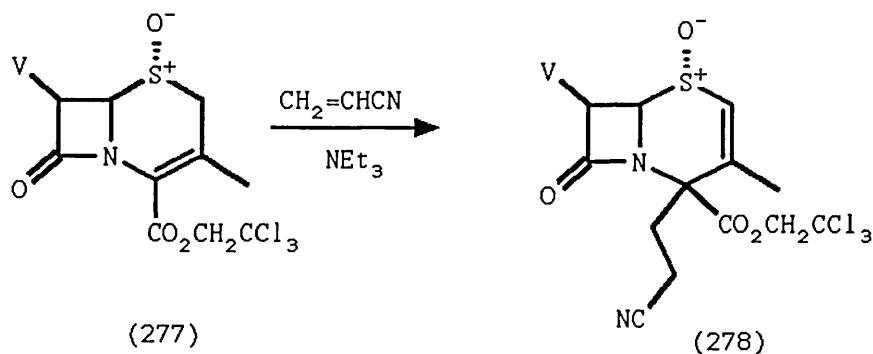
Thus, in summation, reaction of ceph-3-ems with 2 equivalents of LDA followed by quenching gives rise solely to isomerisation of the double bond with no removal of the C-7 proton. When C-4 disubstituted ceph-2-ems are similarly reacted, epimerisation is observed at C-7. This indicates that epimerisation can only occur after isomerisation of the double bond and hence the first equivalent of base removes the NH proton; the second equivalent of base removes the C-2 proton, and further base removes the C-7 proton. These results are in agreement with previous findings and support the mechanism presented above.

2.2.4) Reaction of Ceph-3-em Sulphoxides with LDA.

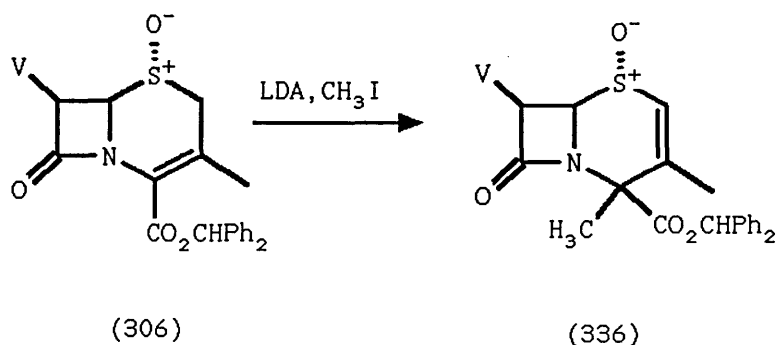
The regiospecificity of cephem alkylation under Michael reaction conditions and with sodium hydride has been outlined in Sections 2.1 and 2.2.1. Some regiospecificity in the reaction of cephem sulphides and β -sulphoxides with LDA and electrophiles has previously been observed^{54, 174}, however a systematic study of the site of alkylation in cephem sulphides, α -sulphoxides and β -sulphoxides has not yet been reported.

It has previously been noted that 2 equivalents of LDA must be used before alkylation of the cephem can occur, and that additional base can cause further deprotonation of the cephem nucleus. For this reason, exactly 2 equivalents of LDA were utilised in the reactions detailed below, and no attempt was made to investigate the regiospecificity of alkylation reactions with excess base since this has previously been found^{159, 162} to occur at C-7, irrespective of the substrate used.

In the preceding section, alkylation of cephem sulphides was shown to give 4-alkylated ceph-2-ems as the sole product. Cephem α -sulphoxides undergo Michael reactions to give 4-substitution only. When



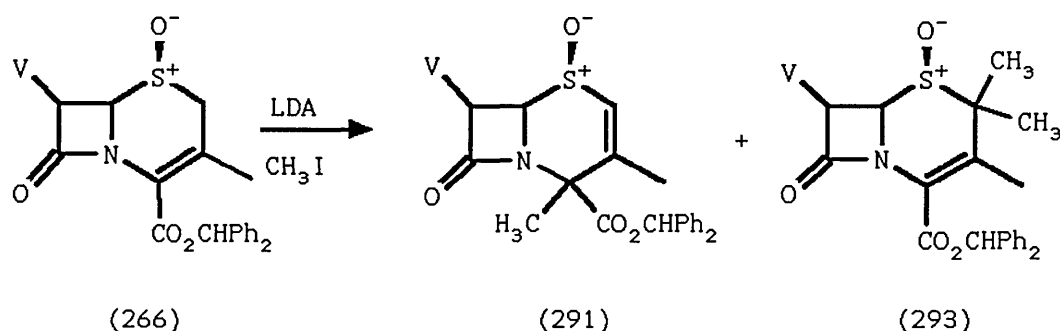
α -sulphoxide (306) is reacted with LDA and iodomethane, a single product (50%) is isolated. UV absorption at 254nm indicated a ceph-2-em structure, and the pmr spectrum showed a doublet at δ 6.18 ($J=1.1\text{Hz}$) coupled with the 3-methyl group which confirmed this structure.



An additional methyl group was also observed in the pmr spectrum, and mass spectroscopic data and elemental analysis confirmed the molecular formula as $\text{C}_{30}\text{H}_{28}\text{N}_2\text{O}_6\text{S}$, thus structure (336) is assigned. As observed with Michael reactions the α -sulphoxide reacts exclusively at C-4, whereas the β -sulphoxide has been found to react solely at C-2^{54, 174} under analogous conditions.

When the isomeric β -sulphoxide (266) is reacted under the same conditions utilised with the α -sulphoxide, two components are isolated from the reaction mixture after chromatography. The R_f values of these two products are very similar to those of (291) and (293) obtained from sodium hydride induced alkylation. The major, less polar compound had a pmr spectrum and melting point identical to those of (293), and UV absorption at 268nm confirmed the ceph-3-em structure. However, the minor component had a UV absorption maximum at 257nm

indicative of a ceph-2-em double bond, similar to that observed for (291). The pmr spectrum indicated a vinylic C-2 proton, coupled with the 3-methyl group of a ceph-2-em and an additional 3 proton singlet was observed which had an identical chemical shift to the 4 β -methyl substituent in (291).



Before trying to rationalise the regiospecificity of alkylation in cephems, it is perhaps useful to summarise the results obtained during this study.

Starting material

		Sulphide	β -sulfoxide	α -sulfoxide
Base used	NaH	4-Methyl (62%)	4-Methyl (62%) 2,2-Methyl (9%)	N/A
	LDA	4-Methyl (60%)	2,2-Methyl (47%) 4-Methyl (14%)	4-Methyl (50%)

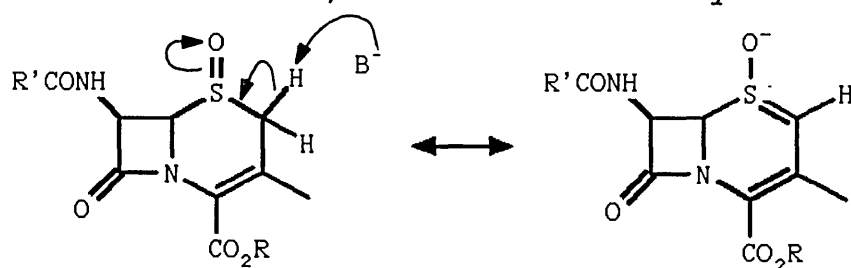
Table 2

A number of factors obviously influence the site of alkylation during these reactions, the most important of these being :

- a) the base used for deprotonation;
- b) steric effects involving the 7 β -side chain and sulphoxide orientation;
- c) relative contributions of the resonance forms of anions at C-2 and C-4;
- d) steric and electronic effects involving the N-5 lone pair;
- e) the nature of the cation.

In the case of sulphide alkylation, both bases give rise to C-4 alkylated products as has previously been observed during Michael reactions. Removal of a C-2 proton by base will result in delocalisation of the negative charge into the C-4 carboxyl group (Scheme 4, Section 2.1). Consequently, the C-4 carbanion canonical form will predominate (since the C-2 carbanion form is not stabilised to the same extent). Alkylation therefore occurs at C-4 and on the β -face due to (d) above.

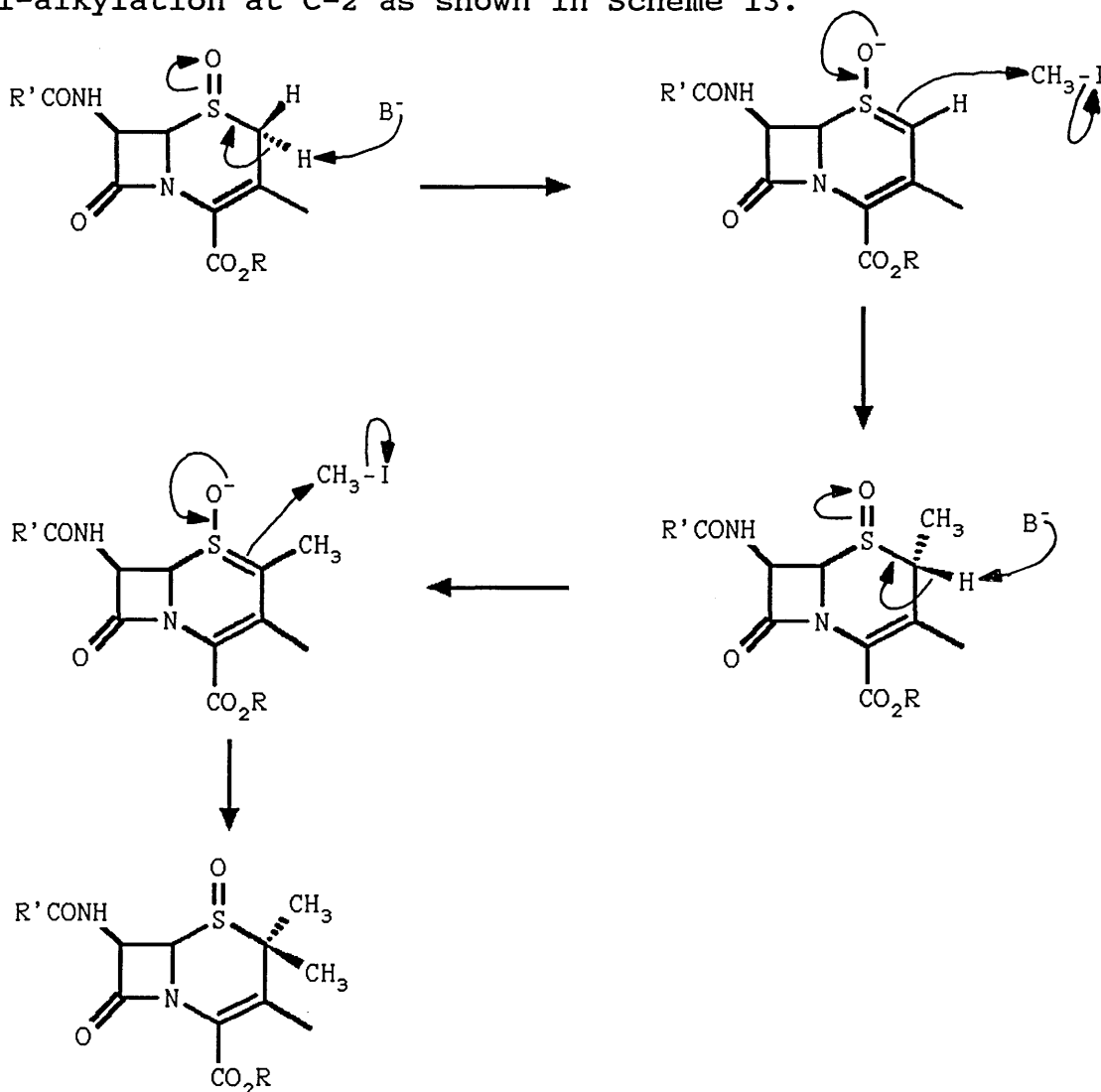
In the α -sulphoxide, factors (b) and (d) appear more important than the enhanced stability of the C-2 carbanion (due to delocalisation of the negative charge into the sulphoxide). Although delocalisation as shown in Scheme 12 will occur, steric hindrance by the



Scheme 12

α -sulphoxide group prevents alkylation at C-2, hence alkylation occurs at C-4.

With the β -sulphoxide the situation is somewhat more complex and the product ratio is dependent on the base employed. In the case of LDA, it appears that the enhanced stability of the C-2 carbanion is the main reason for the predominance of 2-alkylated products. The β -sulphoxide does not hinder alkylations at C-2 on the α -face (unlike the α -sulphoxide) and the second C-2 proton also appears to be readily removed by LDA to give di-alkylation at C-2 as shown in Scheme 13.



Scheme 13

The reaction conditions employed in these LDA reactions (ie: low temperature) will allow kinetic control of the deprotonation step, that is, the most easily removed proton will be removed first. The resulting carbanion may not be the thermodynamically most stable, however the low temperature used means that the deprotonation is essentially irreversible and thermodynamic effects are unimportant. At higher temperatures such as those used in sodium hydride reactions, the thermodynamic stability of the carbanion is an important consideration. Thus, the most easily removed proton (ie: H-2) will result in a C-2 carbanion at -70°C , hence C-2 alkylation is predominantly observed with LDA. However, at 0°C the major product stems from a C-4 carbanion, implying that the C-4 carbanion is thermodynamically more stable than the C-2 carbanion. In addition to thermodynamic and kinetic factors influencing regiospecificity, the nature of the base counter ion may also have some effect on the course of the reaction.

When other electrophiles are used an additional factor in determining the site of alkylation becomes apparent. For example, when the β -sulphoxide (**266**) is reacted with sodium hydride and iodomethane, the 4-alkylated product is primarily obtained. When *t*-butyl bromoacetate is used, only C-2 alkylation is observed. Electronic and, possibly, steric effects due to the electrophile must influence the site of alkylation since all other factors

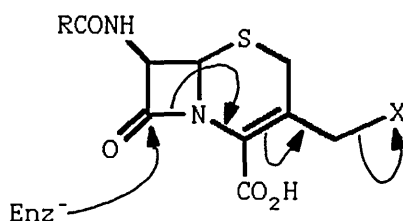
are unchanged.

The choice of alkylating agent has been found to have a significant effect on the site of alkylation in a variety of systems, most notably in the competitive reaction between O- and C-alkylation of enolate anions¹⁸⁷. The effect of alkylating agent has been rationalised by the hard/soft acid and base theory¹⁸⁸ which states that hard Lewis acids will bond to hard Lewis bases, and soft Lewis acids to soft Lewis bases. The relative hardness of Lewis acids and bases is primarily a measure of their polarisability and electronegativity. Thus, enolate anions are comprised of a hard oxygen anion and a soft carbon anion, and the ratio of O- to C-alkylation often reflects the softness of the alkylating agent¹⁸⁹. In general, alkyl iodides are found¹⁸⁷ to produce the greatest yield of C-alkylated products.

In the alkylation of cepheids with iodomethane or *t*-butyl bromoacetate, the regiospecificity observed is possibly attributable to the relative hardness or softness of the alkylating agent. Iodomethane, which would be expected to be the softer of the two alkylating agents, will react with the softest anion, which by interpretation of the results discussed above would appear to be the C-4 anion. On the other hand, *t*-butyl bromoacetate is likely to be a harder alkylating agent and will react with a harder anion which in this case appears to be the C-2 carbanion.

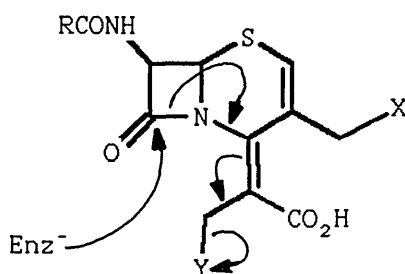
2.2.5) Attempted Synthesis of Cephems with C-4 Exocyclic Double Bonds.

The antibiotic effect of cephalosporins has been accounted for¹⁹⁰ by initial attack at the β -lactam carbonyl, followed by eventual loss of the C-3' leaving group (Scheme 14). The possibility of providing an



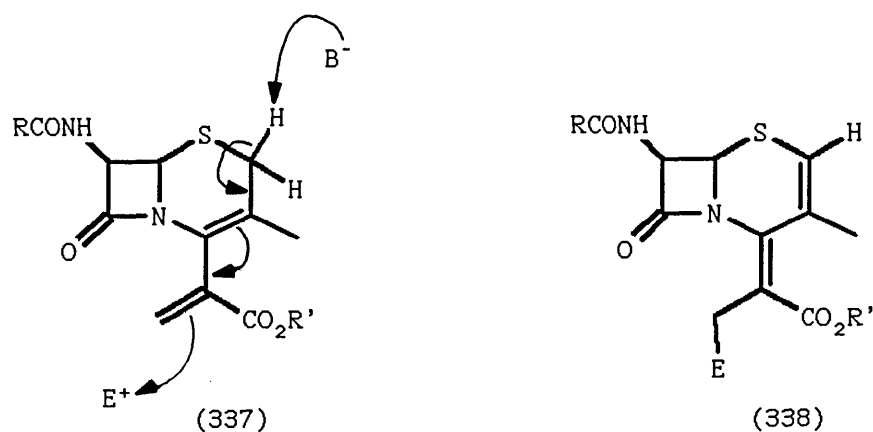
Scheme 14

alternative route for electron flow following cleavage of the β -lactam amide bond was seen to exist *via* the incorporation of a C-4 exocyclic double bond system (Scheme 15).



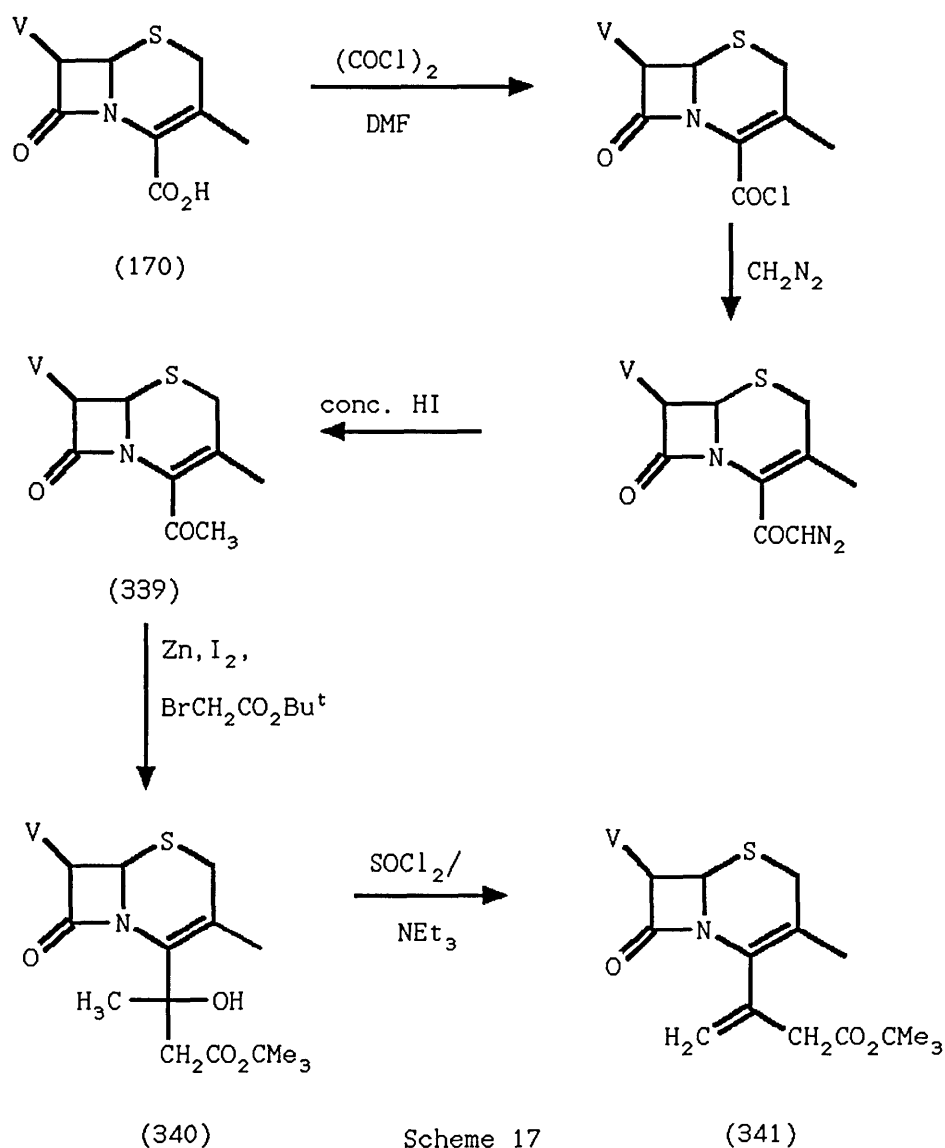
Scheme 15

The most immediate precursor to systems of this type was seen to be the C-4' vinylogous ester (337) which might be deprotonated at C-2, followed by addition of a suitable electrophile (Scheme 16). Compounds similar to

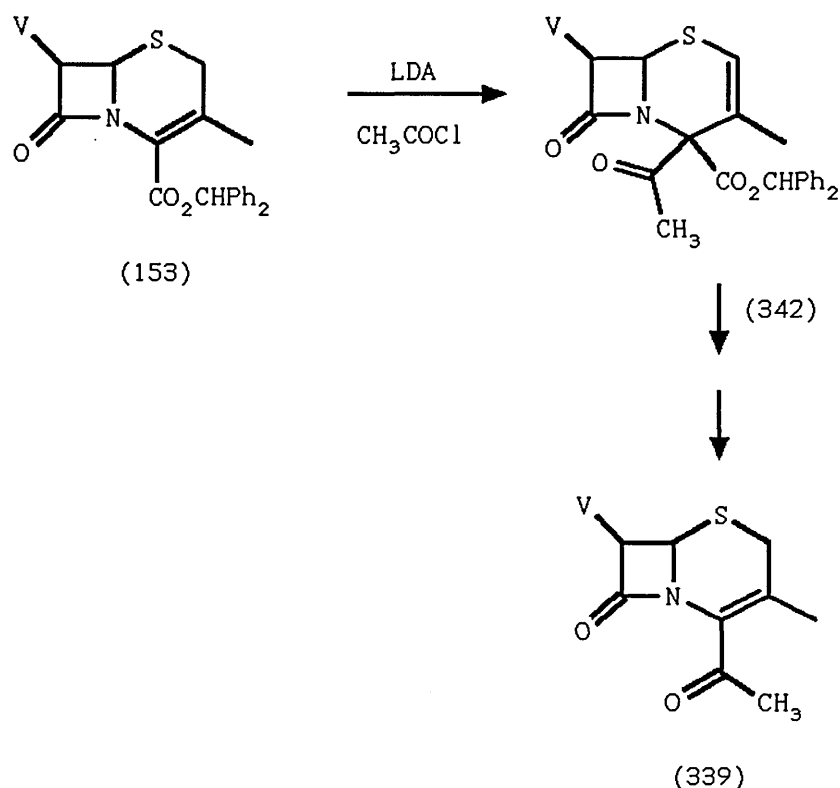


Scheme 16

(337) have been prepared by Balsamo *et al*¹⁹¹ via the following route (Scheme 17).

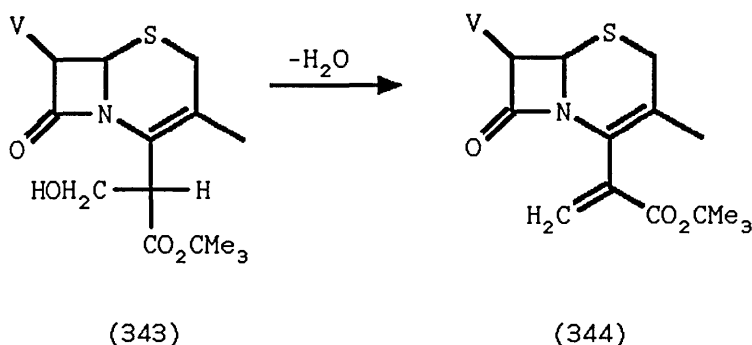


An alternative synthesis of methyl ketone (339) and hence formation of (341) could be visualised from de-esterification and decarboxylation of the C-4 disubstituted ceph-2-em (342), obtained from the acylation of a ceph-3-em using acetyl chloride and base.

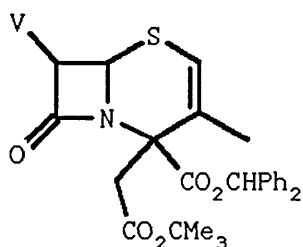


Attempts to acylate the 4-position of cephem (153) with acetyl chloride failed using both NaH and LDA under a variety of conditions. In all cases the starting material was recovered in quantitative yield.

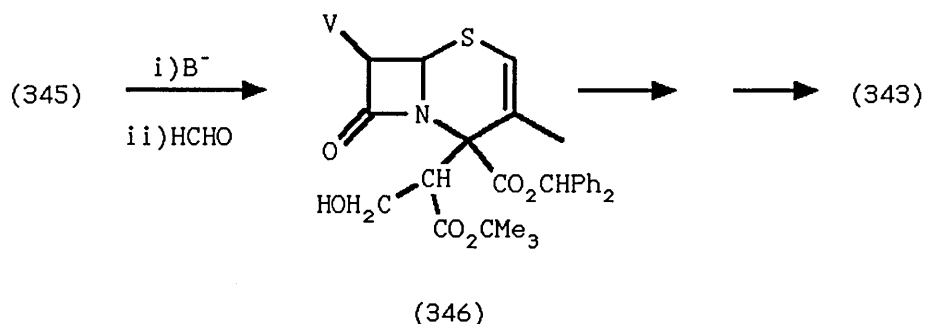
Rather than prepare (341) *via* known methodology, alternative routes to compounds of this type were considered. An isomer of (340), where a hydroxymethyl group is attached to the C-4' carbon *viz* (343), could eliminate water to give the desired exomethylene cephem (344). Consequently, cephem sulphide (153) was reacted



with *t*-butyl bromoacetate. TLC of the reaction mixture indicated a single product which was isolated after column chromatography. Elemental analysis denoted a molecular formula of $C_{35}H_{36}N_2O_7S$ consistent with the incorporation of one *t*-butyl acetate group, and a molecular weight of 628 was also observed. The pmr spectrum indicated a ceph-2-em structure as expected, with coupling between the H-2 proton and the C-3 methyl group ($J=1.2\text{Hz}$). Additionally an AB quartet due to the α -protons of the *t*-butyl acetate group and a 9 proton singlet due to the ester further confirmed the incorporation of a single ester residue.

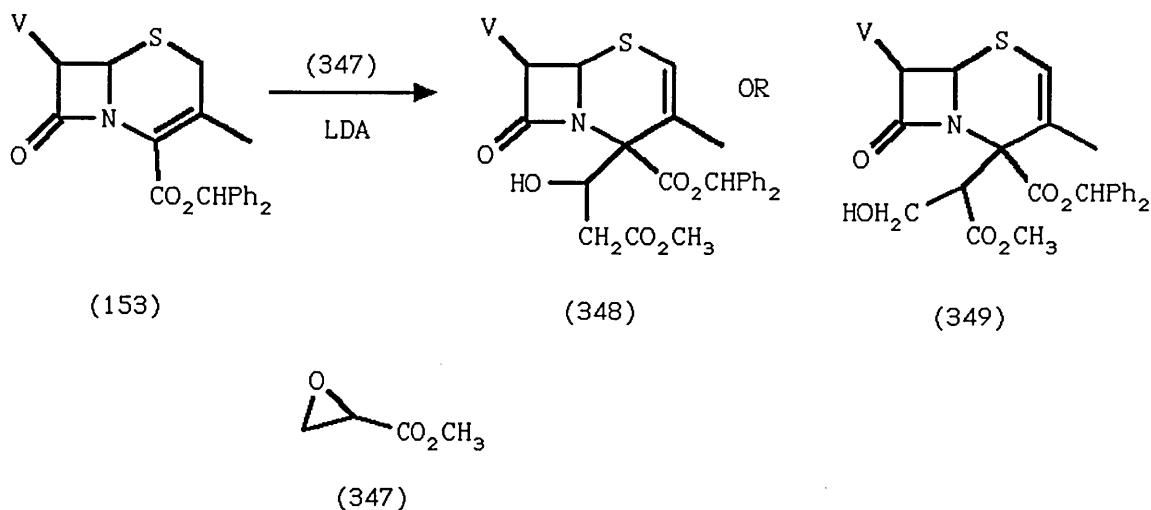


Deprotonation of the C-4' position, followed by addition of formaldehyde would give the hydroxymethyl cephem (346), from which the original C-4 carboxyl group could be removed as detailed in Section 2.1.4 to produce the C-4 monosubstituted ceph-3-em (343).



As a model reaction to determine the feasibility of removing this proton, ceph-2-em (345) was reacted with two equivalents of base and one equivalent of iodomethane. No reaction was observed by tlc, however the reaction mixture was worked up as usual to afford unreacted starting material (345) in quantitative yield. It is surprising that no alkylation is observed since the possibility still exists of removing the C-7 proton.

Since it had not proved possible to synthesise (346) by this route, it was envisaged that reaction of a cephem C-4 carbanion with a glycidic ester would result in ring opening of the oxirane system to give either (348) or (349), depending on the mechanism of ring opening. Methyl glycidate (347) was prepared *via*



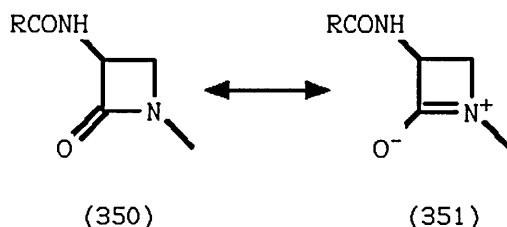
Methyl glycidate (347) was prepared *via* oxidation of methyl acrylate with *m*-CPBA¹⁹² in refluxing dichloroethane with BHT present as a polymerisation inhibitor. The crude oxirane was purified by Kugelrohr distillation to afford a waxy white solid. Attempts to react (347) with a cephem carbanion were unsuccessful, and only Δ -2/ Δ -3 isomerisation was observed.

At this stage, molecular modelling studies of exocyclic double bond systems had been completed (Section 2.2.6). These studies suggested that exocyclic double bonds attached to the 2-position may result in compounds with a greater likelihood of antibacterial activity. Consequently, due to the problems encountered in preparing compounds of type (338), further research was directed towards reactions of the C-2 position.

2.2.6) Molecular Modelling of Cephem Derivatives.

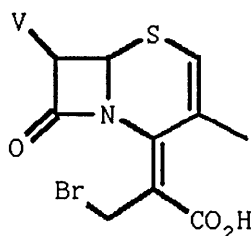
In general, for a β -lactam to be biologically active it must be sufficiently structurally similar to natural substrates to be recognised by cell wall enzymes. In addition, the β -lactam bond must be reactive enough to allow rapid acylation of the enzyme of the active site.

The β -lactam ring can be considered¹⁹³ to have resonance forms (350) and (351) which indicate that the C=O bond should be longer and the C-N bond shorter than for a normal C=O or C-N bond. For this theory to hold,



the three atoms connected to the β -lactam nitrogen must be coplanar. This has been shown¹⁹⁴ not to be the case due to steric reasons and consequently, the C=O bond in a β -lactam is shorter than a normal amide bond¹⁹⁴, and the C-N bond longer. In simplistic terms, active penicillins and cephalosporins have a greater degree of C=O double bond character than C=N double bond character. Therefore, the smaller the ratio of C=O bond length to C-N bond length, the more reactive the system. In practice, this bond ratio is only a very rough guide to activity since no account is taken of the stereochemistry of the molecule. It has previously been observed¹⁹⁵ that bicyclic β -lactams which are biologically active have a C=O/C-N bond ratio in the range 0.835-0.855.

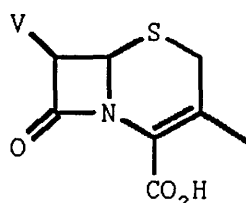
The cephem acid (352) was modelled using Chem-X^{196, 197}



(352)

software by modification of an existing cephem nucleus derived from X-ray crystallographic data. Energy minimisation of the molecule with the semi-empirical

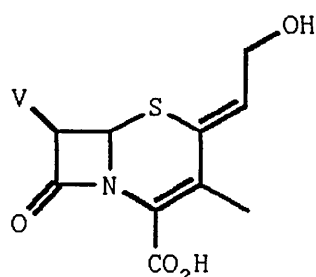
AMPAC package produced the energetically most favourable conformation of the molecule. The C=O and C-N bond lengths were then determined. In this case a ratio of 0.847 was observed which is towards the upper limit of the expected range for activity. An identical value was obtained when cephem acid (170) was modelled, however



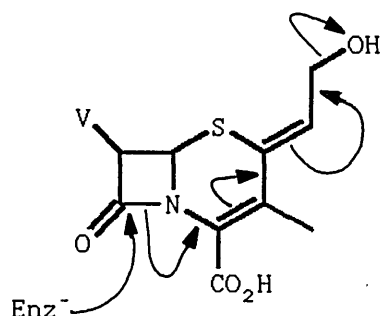
(170)

the large difference in stereochemistry around the C-4 position will affect the binding of (352) with transpeptidase enzymes.

In addition to the C-4 exocyclic double bond system (352), it was decided to model the C-2 exocyclic double bond system (353), a cephem analogue of clavulanic acid. Again, an alternative electron flow can be visualised, though in this case the stereochemistry around the C-4 position is not affected.



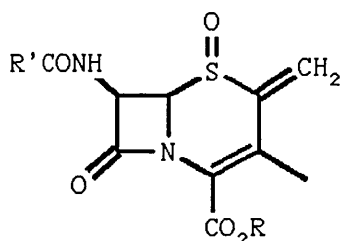
(353)



When this molecule was modelled, a C=O/C-N bond ratio of 0.841 was observed which is closer to the values obtained for β -lactams which are known to be active.

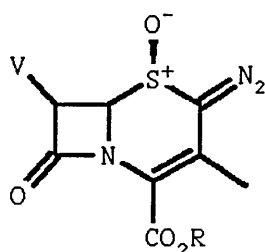
2.3) REACTIONS AT C-2 TO FORM EXOCYCLIC DOUBLE BONDS.

The simplest cephem containing a C-2 exocyclic double bond, viz the 2-exomethylenecephem (42), was prepared from the corresponding sulfoxide by a Mannich reaction^{49, 50}. Other simple exocyclic double bond

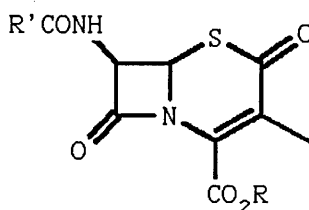


(42)

systems synthesised include the 2-diazocephem (354)¹¹⁴ and the 2-oxocephem (355)^{198, 199}. The diazo sulfoxide



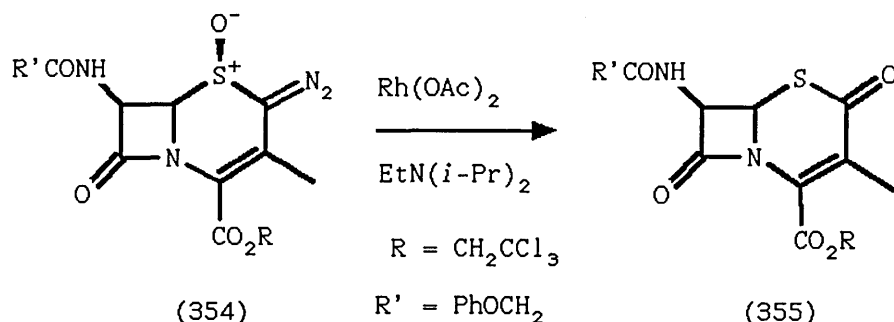
(354)



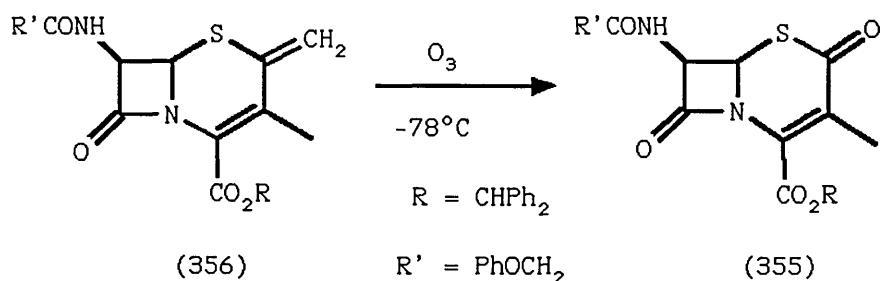
(355)

(354) was prepared by diazo transfer from tosyl azide to the cephem sulfoxide in the presence of triethylamine and has proved to be a versatile intermediate leading to a variety of 2-substituted cephems when reacted with electrophiles. Cephem (354) has also been prepared by diazo transfer with picryl azide¹⁹⁸. These authors also

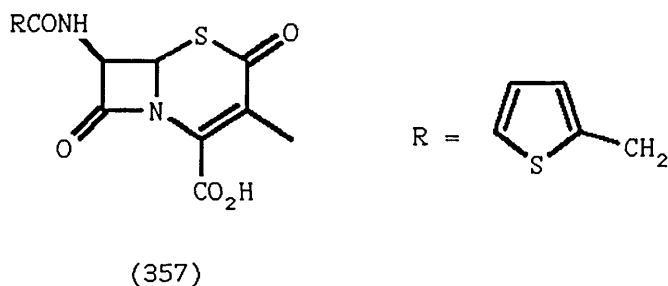
reported that reaction of the diazocephem with rhodium acetate produced the 2-oxocephem (355) in low to moderate yield. 2-Oxocephems have also been prepared¹⁹⁹



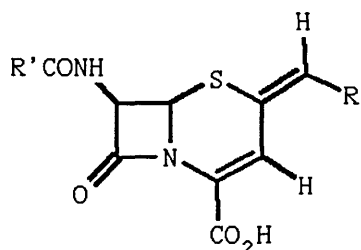
by ozonolysis of the corresponding 2-exomethylenecephem at low temperature. Biological activity of the



2-oxocephem was found to be poor for a variety of C-7 and C-3' substituents. The 2-oxo analogue of cephalothin (357) was found to have MICs of up to 2 orders of magnitude higher than the unsubstituted cephem against most organisms¹⁹⁹.



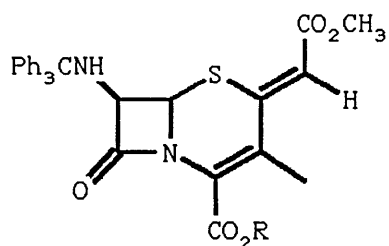
Cephems with a C-2 exocyclic double bond such as (358), which do exhibit biological activity, have been prepared via total synthesis by Woodward and co-workers²⁰⁰. The



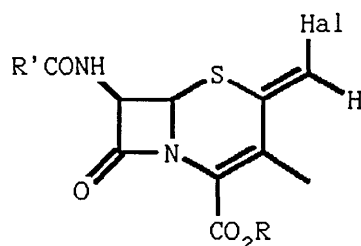
(358)

R = alkyl, (substituted)aryl

formation and reactions of the C-2 exocyclic double bond systems (53) and (43) have previously been discussed in Section 1.3.



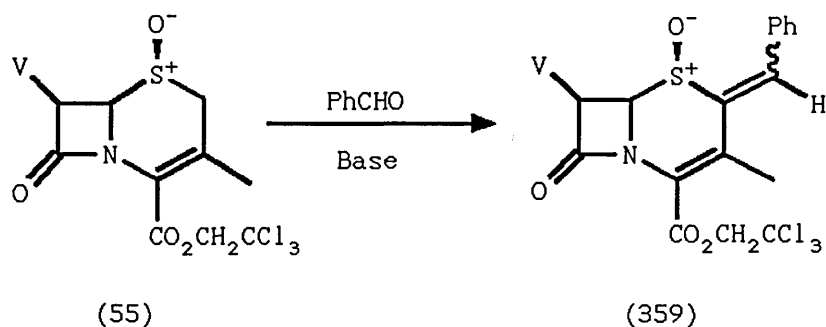
(53)



(43)

2.3.1) Reactions of the C-2 Methylene Group.

The C-2 protons in cephem sulfoxides are reasonably acidic and can be removed by base to produce 2-substituted products (see Section 1.3). Under certain circumstances both protons can be removed¹⁶² and subsequently it was considered likely that cephem sulfoxides would undergo a base-catalysed condensation similar to the Knoevenagel reaction²⁰¹. Thus, treatment of sulfoxide (55) with benzaldehyde in the presence of base was expected to result in the C-2 exocyclic double bond system (359), similar to those synthesised *via* total synthesis²⁰⁰. Knoevenagel reactions have

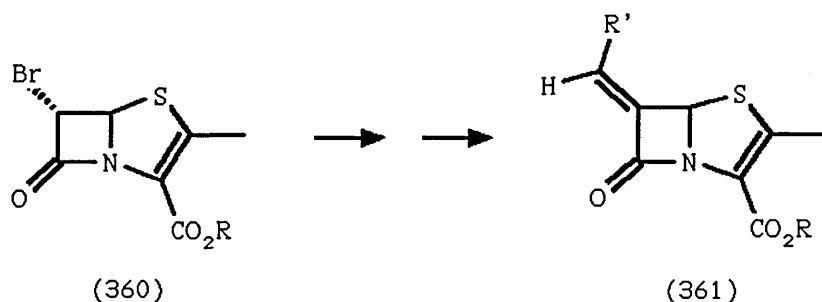


previously been reported using a wide variety of bases and reaction conditions. Triethylamine was initially selected as base and the reaction was attempted in acetonitrile at both room temperature and 0°C, and also in refluxing toluene. In each case the solution gradually darkened and tlc indicated consumption of starting material, however no product was detected. After reaction overnight, the infrared spectrum of the reaction mixture showed complete lack of any β -lactam carbonyl. The formation of benzoic acid from air oxidation of benzaldehyde, and production of water during the reaction could both contribute to decomposition of the starting material. The reaction was repeated in an inert atmosphere using refluxing toluene and a Dean & Stark separator with NEt_3 as base, however no difference was noted in the reaction, with degradation of the sulphoxide observed as before. Decreasing the reaction temperature (refluxing acetonitrile) and the addition of 4A molecular sieves helped to prevent decomposition of (55), however no incorporation of the aldehyde was observed. The use of stronger bases such as NaH and LDA also proved

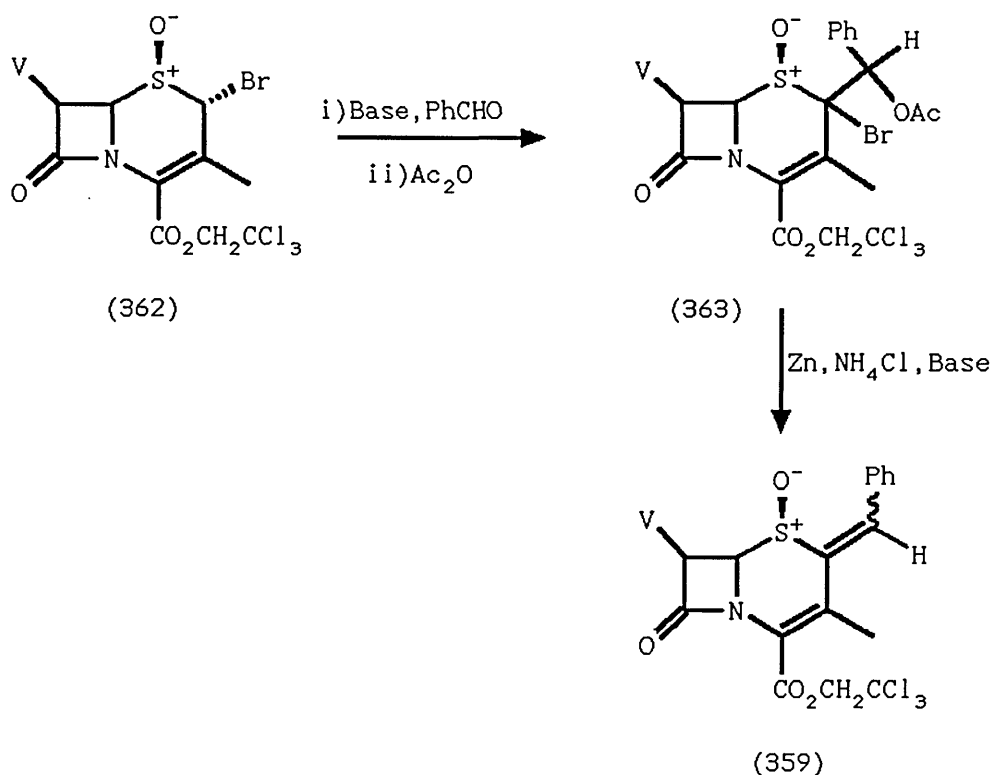
unsuccessful.

An alternative route to exocyclic double bond systems of this type may lie in the Beecham process²⁰² for preparing penems with a substituted methylene group at the 6-position (361) from 6 α -bromopenems (360).

Compounds of general structure (361) have been found to be potent β -lactamase inhibitors. Thus, 2-bromocephem

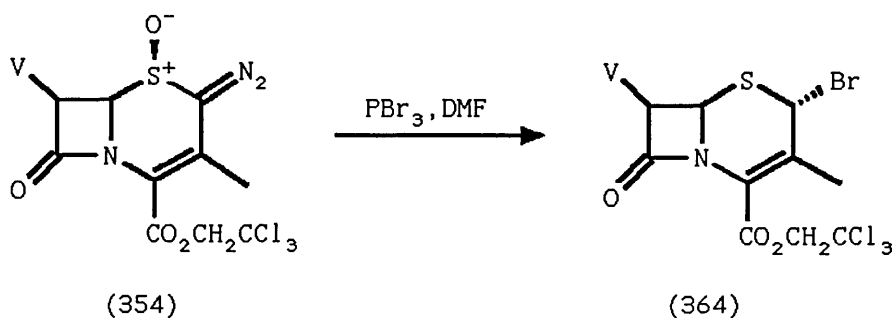


(362) could be deprotonated and the resulting carbanion treated with an aldehyde, followed by trapping of the intermediate alcohol as the acetate (363) which could then be converted into (359).



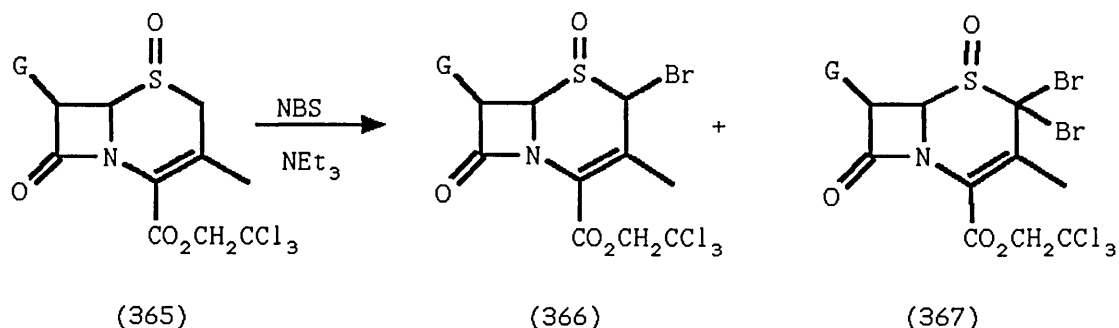
2.3.2) Attempted Reactions of 2-Bromocephems.

The 2 α -bromocephem (364) has previously been prepared by treatment of diazosulphoxide (354) with PBr₃ in DMF¹¹⁴. Several methods for the preparation of α -bromo-



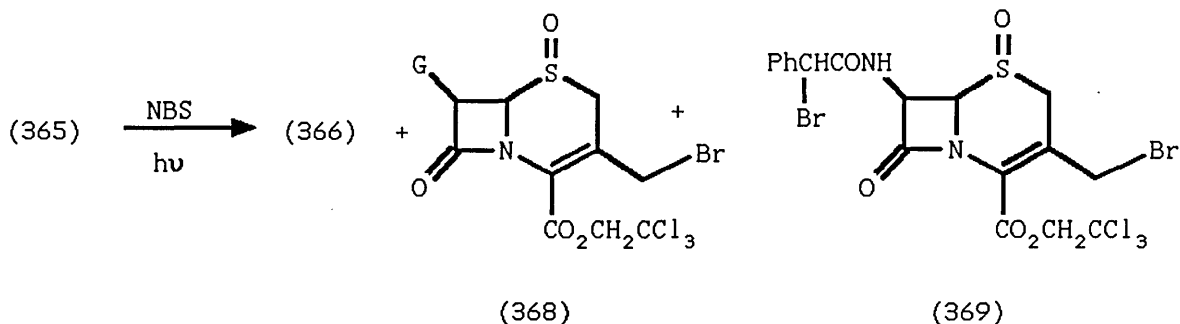
sulphoxides have been reviewed²⁰³, and of these, N-bromosuccinimide (NBS)²⁰⁴ and NBS/bromine²⁰⁴ were selected for synthesis of (362).

In the cephalosporin series, 2-bromocephems are often formed as intermediates or by-products in the synthesis of 3-bromomethylcephems^{205,206}. In these cases, bromination is carried out using NBS in the presence of an organic base such as NEt₃. Thus, cephem (365) reacts with NBS and NEt₃ to give the 2-bromocephem (366) as the major product along with some 2,2-dibromocephem (367)²⁰⁵.



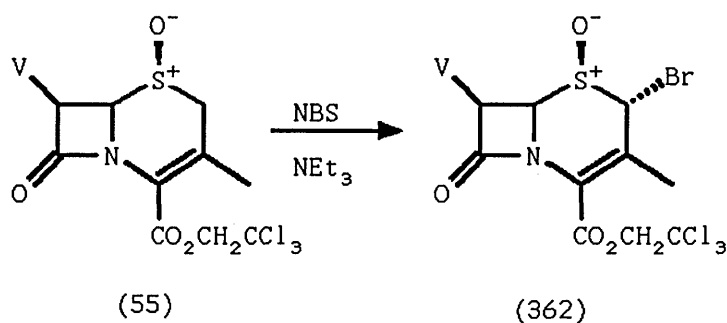
In contrast, direct photo-initiated bromination²⁰⁷ of (365) in the absence of base gives rise to a mixture of the 2-bromocephem (366), 3-bromomethylcephem (368) and

the dibromocephem (369). The proportion of each was found to be dependent on the reaction conditions. In



the presence of triethylamine, one of the C-2 protons in (365) is readily removed to form a carbanion which is brominated. In the absence of base, free radical bromination will occur at all allylic and benzylic sites within the molecule, as expected with NBS. The ratio of compounds will depend on the reaction temperature, since at higher temperatures the thermodynamic product(s) will predominate whereas at lower temperatures the kinetic product(s) will be produced.

When sulfoxide (55) is treated with NBS in the presence of NEt_3 , a single product is observed by tlc with no starting material left after 30 minutes. The reaction mixture was chromatographed and the product isolated as a pale yellow foam. The mass spectrum indicated molecular ions at 572 and 574 corresponding to the incorporation of one bromine atom. The pmr spectrum showed that the C-3 methyl group was intact, hence bromination had not occurred at C-3'. The AB quartet normally present for the C-2 protons was not observed, and a singlet integrating for 1 proton at $\delta 5.15$ was assigned to the C-2 proton in (362). The possibility

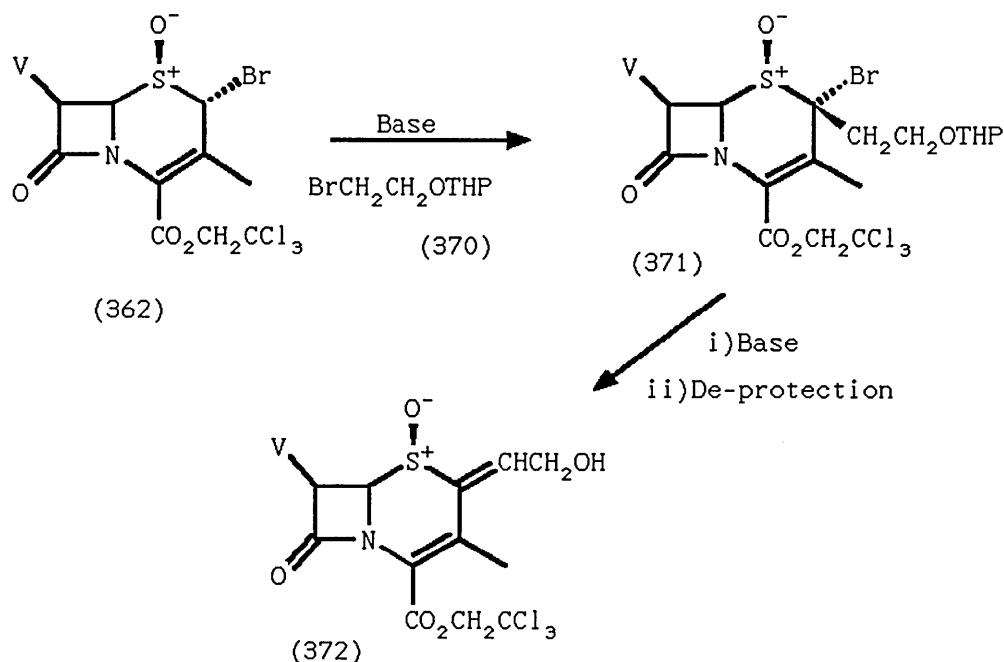


exists for bromination on either the α - or β -face at C-2. In the parent sulfoxide (55), long-range coupling is observed between H-6 α and H-2 α ¹⁶⁴. No such coupling was present in the pmr spectrum of (362), hence bromination occurs on the α -face of the molecule. Steric hindrance due to the 7 β -side chain and the β -sulfoxide make the β -face difficult to attack, hence deprotonation and subsequent bromination occur on the less hindered α -face.

When sulfoxide (55) was brominated with a mixture of NBS and molecular bromine in the presence of pyridine, the same product (362) was obtained. This reagent has been reported²⁰⁴ to afford α -bromosulfoxides in higher yield than either bromine or NBS separately. The bromination of (55) with NBS/NEt₃ had afforded the 2-bromocephem in 90% yield hence it was not expected that alternative brominating agents would greatly increase this yield. In reality, NBS/Br₂/pyridine resulted in formation of 2-bromocephem (362) in 60% yield. This is probably due to the decreased ability of pyridine to remove the C-2 proton (which has also been noted in Michael reactions of ceph-3-ems²⁰⁸).

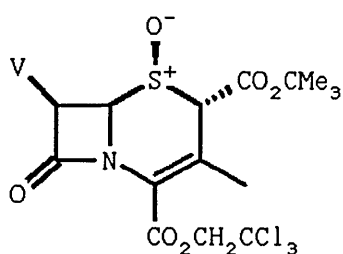
Attempts to de-oxygenate the bromocephem sulphoxide to yield the 2-bromocephem sulphide, previously prepared by Bremner and Campbell, failed with both PBr_3 and $\text{KI}/\text{CH}_3\text{COCl}$. Spry has also noted⁴² that 2 α -chlorocephem sulphoxides fail to de-oxygenate under these conditions.

The use of the 2 α -bromocephem (362) as a precursor of cephems with exocyclic double bonds at the 2-position has not proved successful. Deprotonation of the remaining C-2 proton in (362), followed by addition of an electrophile was expected to result in C-2 disubstituted cephems. Thus, reaction with base and O-protected 2-bromoethanol (370) would result in (371) from which HBr could be eliminated and the hydroxyl group deprotected to give the protected cephem analogue of clavulanic acid (372).

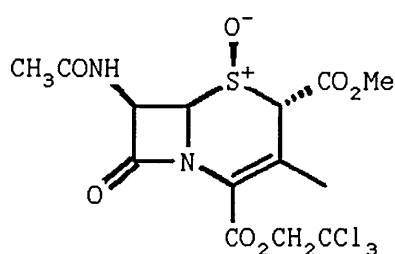


Since organic bases such as triethylamine and Triton B have previously been found to deprotonate the C-2 position in cephem sulphides and sulphoxides, these

bases were initially used to try and deprotonate (362). When (362) was stirred in methyl vinyl ketone in the presence of NEt_3 , no reaction was observed by tlc after several hours. Continued stirring at room temperature resulted in extensive degradation of the starting material to non β -lactam containing products. The failure of weak organic bases to catalyse the reaction prompted the use of stronger bases. Thus, reaction of (362) with both sodium hydride and lithium diisopropylamide followed by addition of iodomethane as a test electrophile also led to degradation of the substrate, resulting in very polar non β -lactam containing products. When (362) was stirred with triethylamine in the absence of an alkylating agent a similar result was obtained. These results suggest that anion formation probably occurs, but decomposition is the preferred pathway, rather than alkylation. Spry has also found⁵⁵ that C-2 α -substituted cephem sulfoxides (59) and (373) failed to alkylate at C-2.



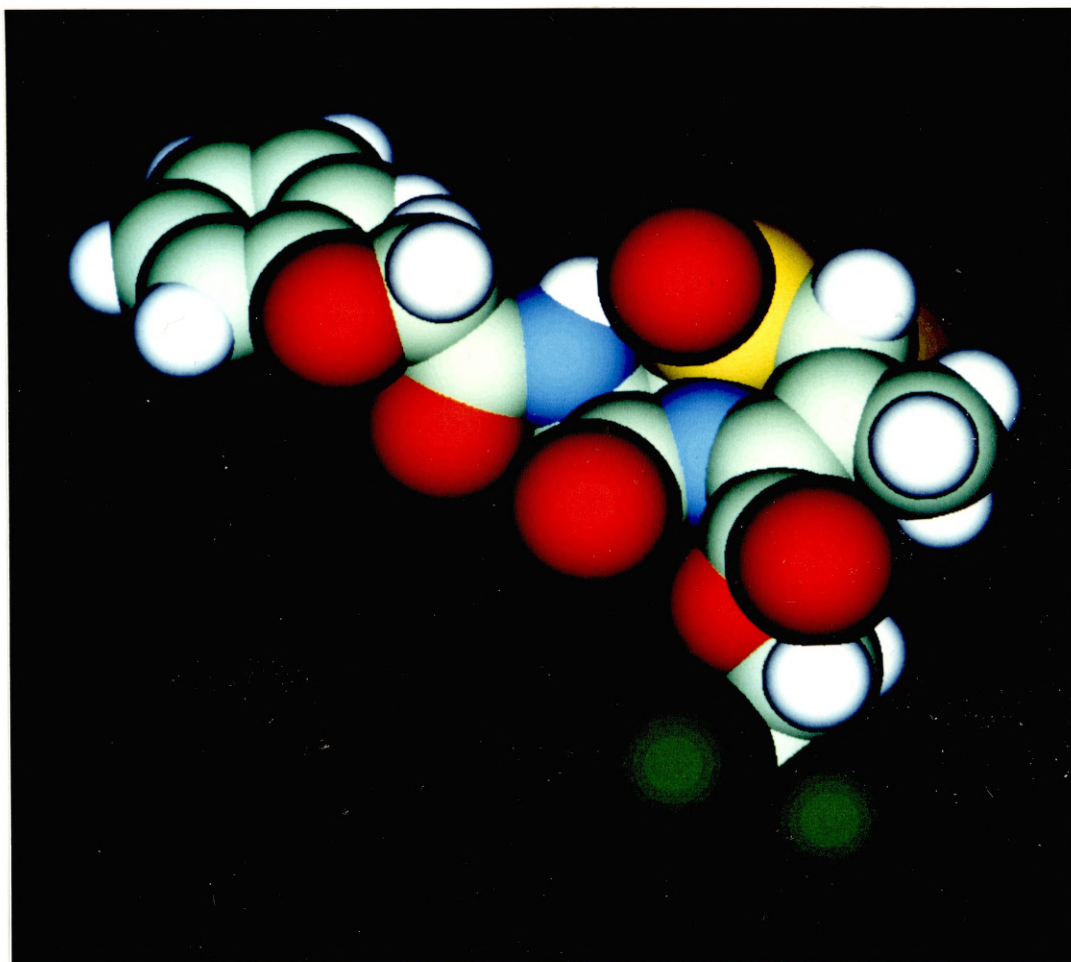
(59)



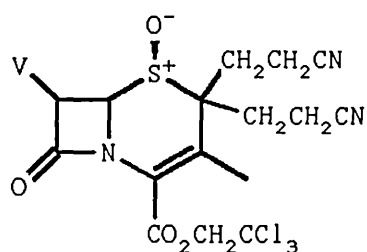
(373)

Construction of a space-filling model of the 2 α -bromocephem sulfoxide (362) shows that the 2-position experiences considerable steric hindrance due to the β -sulfoxide on one face and the α -bromine on the

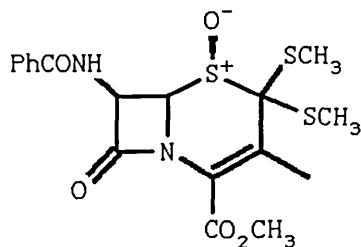
other. Molecular modelling of (362) with Chem-X^{196,197} followed by energy minimisation resulted in a conformation with similar steric hindrance at C-2, and this is illustrated in the photograph below.



Previous reports^{114,159,174} have detailed the synthesis of the 2,2-disubstituted cepheims (276) and (310). In these cases initial deprotonation, followed



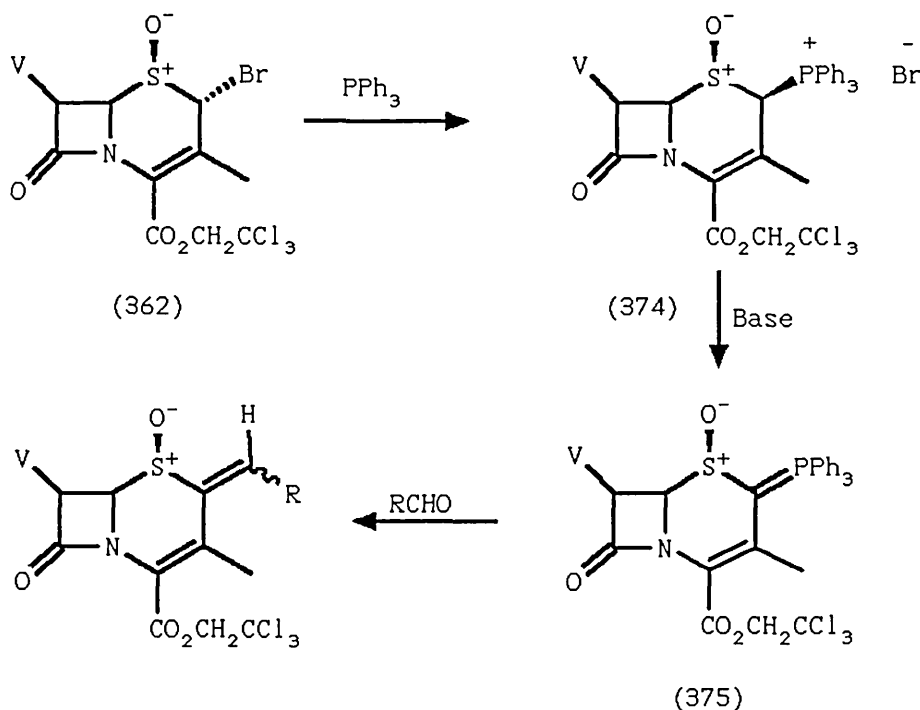
(276)



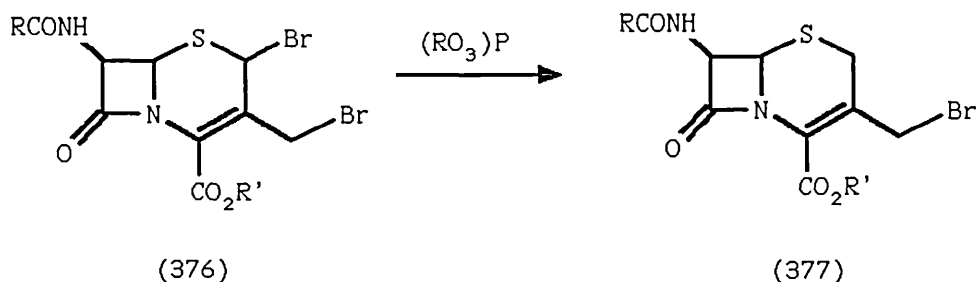
(310)

by alkylation or methylthiolation will occur on the α -face to give the 2 α -monosubstituted cepheids (which are obtained as additional products in low yield) followed by removal of the C-2 β -proton by further base. The increased structural flexibility at C-2 in (276) and the decreased size of the 2-substituent of (310) do not prevent removal of the second C-2 proton.

Reaction of (362) with triphenylphosphine to give phosphonium salt (374) was expected to provide an alternative entry into 2-exocyclic double bond systems via phosphorane (375). The β -orientation of the



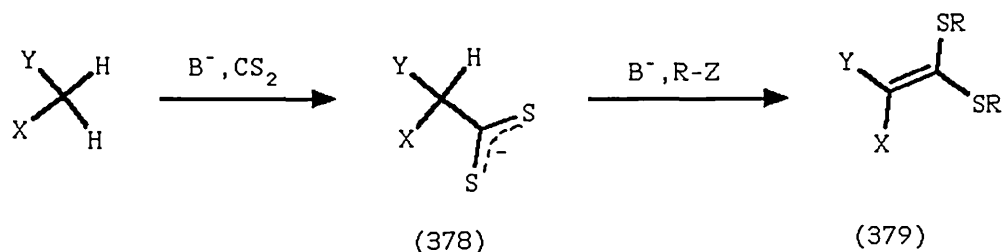
sulphoxide function in (362) will prevent nucleophilic attack by PPh_3 on the C-2 position (since an $\text{S}_{\text{N}}2$ mechanism is involved) and consequently when the 2 α -bromocephem was reacted with PPh_3 under a variety of conditions, degradation of the starting material to afford non β -lactam containing products was observed. Trialkyl phosphites have previously been employed²⁰⁶ for the debromination of 2-bromo-3-bromomethylcephems (376)



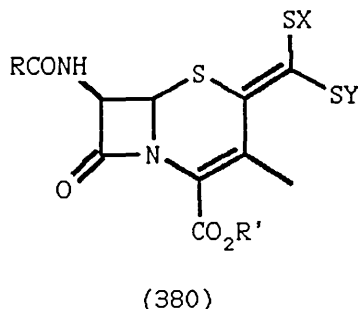
and triphenylphosphine is itself a well-known debrominating agent²⁰⁹ hence it is possible that in the absence of formation of phosphonium salt (374), PPh_3 may debrominate the starting material and lead to decomposition products.

2.3.3) Reaction of Cepems with Heterocumulenes.

Carbon nucleophiles react with heterocumulenes such as carbon disulphide in the presence of base and alkylating agents to give, in the first instance, anion (378). Further base removes the second proton from the active methylene group and alkylation leads to a ketene dithioacetal (379)²¹⁰.



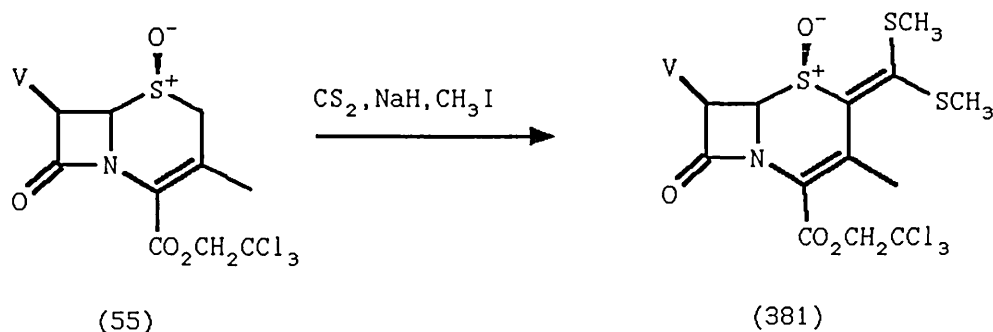
Thus, the possibility exists for the synthesis of completely novel C-2 substituted cephem of type (380).



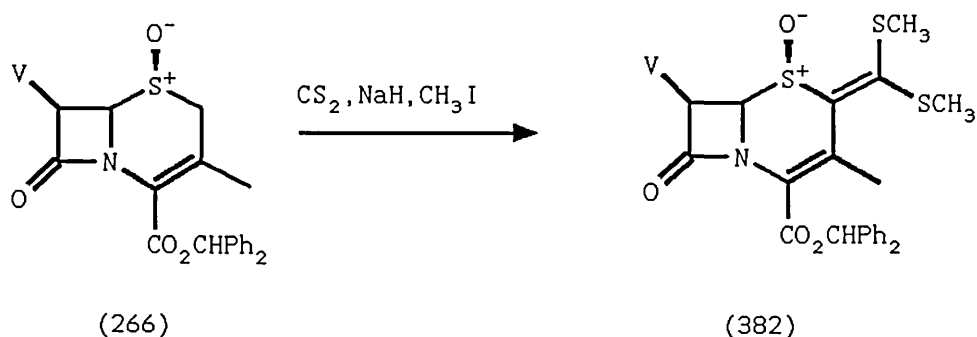
Reactions of carbon nucleophiles with carbon disulphide have recently been reviewed²¹¹. The choice of base in reactions of this type is of great importance since the base must be able to deprotonate the reactant, but must not react with carbon disulphide. Sodium hydride in DMSO affords good yields of dithioacetals from active methylene groups under mild conditions²¹¹ so this base was initially selected for investigation.

When cephem sulphoxide (55) was stirred with sodium hydride in DMSO/carbon disulphide, the solution took on an intense red colouration which turned bright yellow after addition of iodomethane. Complete conversion of the starting material to a less polar product was observed by tlc, and after work-up and chromatography a bright yellow solid was isolated. The pmr spectrum did not contain any C-2 protons, and an additional singlet

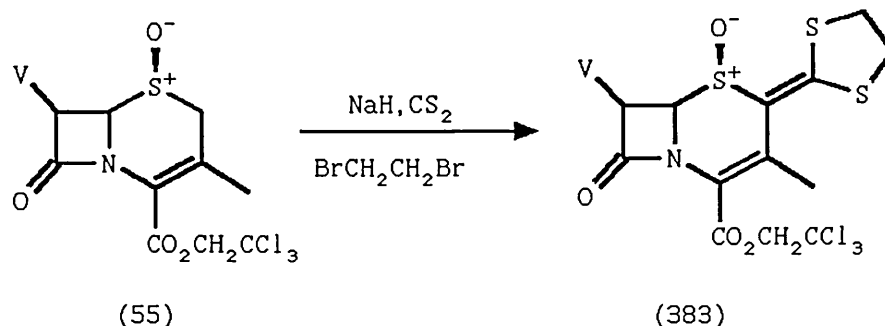
integrating for 6 protons was observed. All other signals were as expected and the infrared spectrum showed retention of the β -lactam carbonyl stretching frequency at 1796cm^{-1} . The mass spectrum indicated a molecular mass of 600, and elemental analysis confirmed the structure as $\text{C}_{21}\text{H}_{21}\text{N}_2\text{Cl}_3\text{O}_6\text{S}_3$. Structure (381) is therefore ascribed to this product and the yield for the reaction is 61%. In an analogous manner, cephem (266)



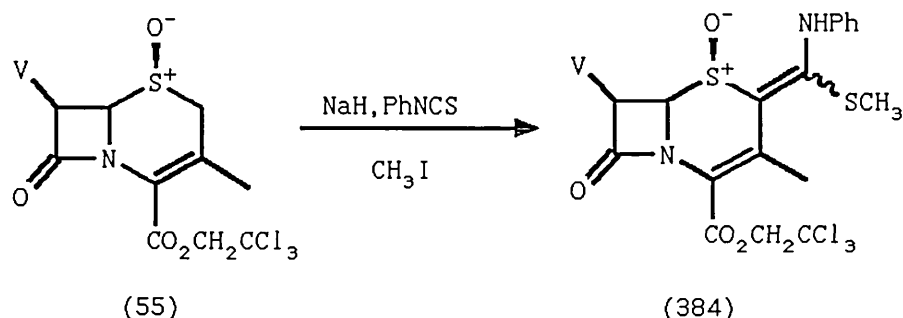
reacted with sodium hydride in DMSO/ CS_2 followed by addition of iodomethane to afford ketene dithioacetal (382) in 60% yield. In order to expand the scope of



this reaction, alternative electrophiles and heterocumulenes were utilised. When sulfoxide (55) was stirred with sodium hydride in DMSO/ CS_2 followed by addition of 1,2-dibromoethane, cyclic disulphide (383) was isolated in 42% yield. The pmr spectrum indicated



incorporation of four additional protons, present as a complex multiplet. The mass spectrum confirmed the predicted molecular weight of 598, and elemental analysis was also consistent with structure (383). When carbon disulphide is replaced by phenyl isothiocyanate, the possibility exists for forming either the S- or N-alkylated products and, in addition, E- and Z-isomers of both products could be produced. Reaction of (55) with NaH in phenyl isothiocyanate followed by addition of iodomethane proceeded smoothly to give a yellow solution containing only one component (tlc). The pmr spectrum of the isolated product (42%) had an additional phenyl group and methyl group. The presence of an additional NH proton in the spectrum indicated that S-methylation had occurred to give (384). Similarly the

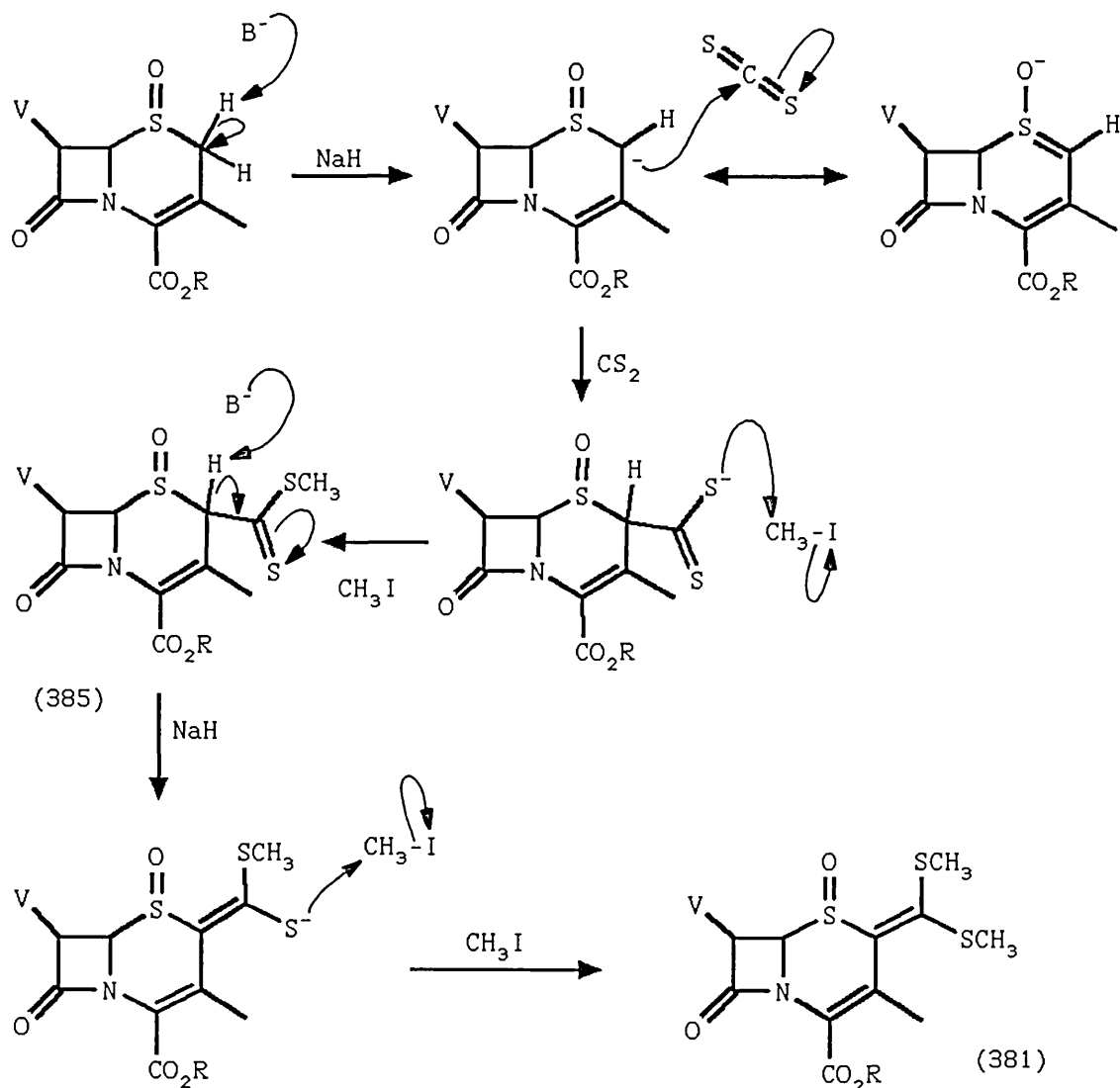


infrared spectrum showed no peaks due to S-H stretch at $2500-2600\text{cm}^{-1}$. The pmr spectrum did show some

duplication of signals and this is attributed to the presence of both E- and Z-isomers of the product. Mass spectroscopy and elemental analysis were both consistent with structure (384).

Under the conditions employed in these reactions with heterocumulenes it is probable that the base used to deprotonate the cephem is the highly reactive sodium methylsulphinylmethide (or dimsyl anion) rather than hydride anion. Corey and Chaykovsky²¹² prepared this reagent by addition of NaH to DMSO at 65-70°C. Even at room temperature, formation of dimsyl anion should be significant. In an alternative solvent the reaction would be expected to occur either in lower yield or possibly to form different products as a result of deprotonation by hydride anion. When the reaction between (55), carbon disulphide and sodium hydride was carried out in THF rather than DMSO, addition of iodomethane resulted in a bright yellow solution as before. Only one component was observed on tlc which had an identical R_f to (381), however more baseline material was observed in this case. Isolation of the product afforded a yellow solid identical (tlc,mp,pmr) to (381). The yield in this instance was, however, much reduced compared to that observed in DMSO solution (25% against 61%). This decreased yield suggests that although sodium hydride can itself act as base in these reactions, the dimsyl anion is preferred due to its increased basicity.

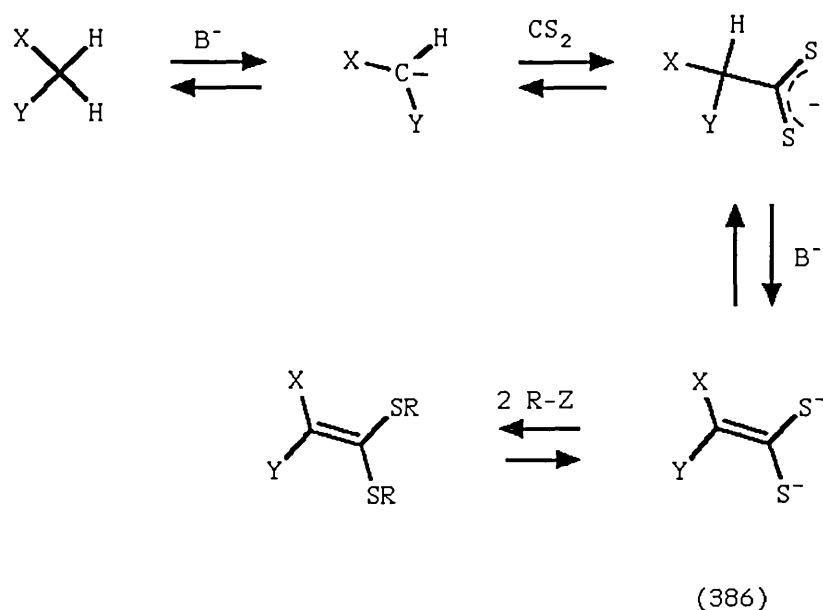
The reactions of cepheids with base and carbon disulphide involve 2 molar equivalents of both base and alkylating agent, in the presence of excess CS_2 . The reaction is believed to proceed *via* the dithioester (385) as shown in Scheme 18. If a second equivalent of base was not present, it was expected that this dithioester could be isolated.



Scheme 18

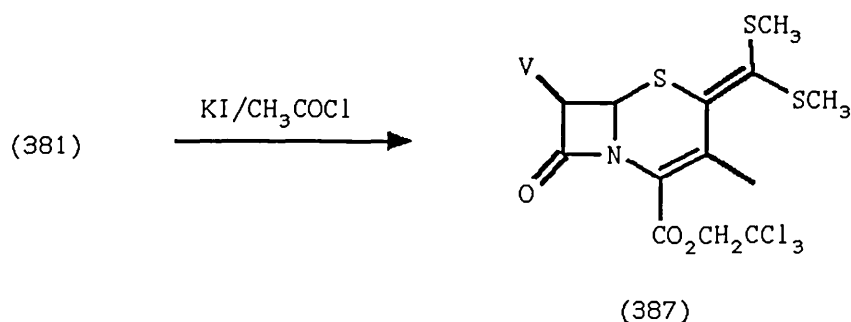
When the reaction of (55) with NaH, CS_2 and CH_3I was repeated using one equivalent of base and electrophile,

tlc indicated formation of (381) along with unreacted starting material. The two components were isolated in approximately equal amounts after chromatography. This suggests that either dithioester (385) is not involved in the reaction pathway, or that it is too reactive to be isolated and consequently reacts to produce dithioacetal (381). It has been suggested²¹¹ that for reactions of this type, the mechanism involves dithiolate anion (386) which is subsequently alkylated to afford dithioacetals as shown in Scheme 19. This mechanism would account for the absence of (385) in the reaction involving only 1 equivalent of base and electrophile.

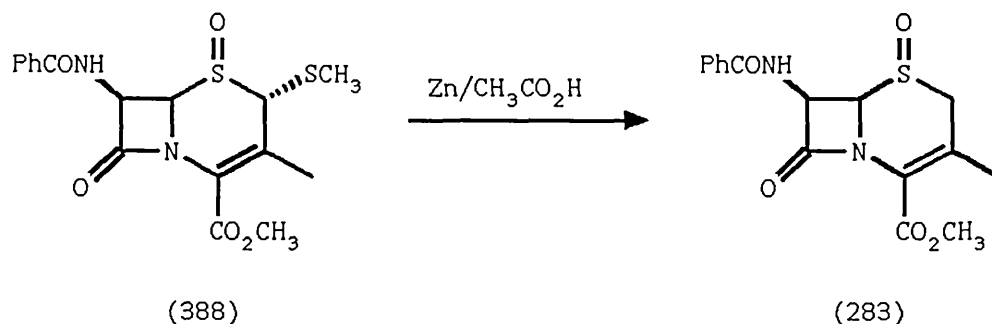


Scheme 19

De-oxygenation of sulphoxide (381) proceeded smoothly with acetyl chloride/potassium iodide to afford the corresponding sulphide (387) in 81% yield. Attempts to



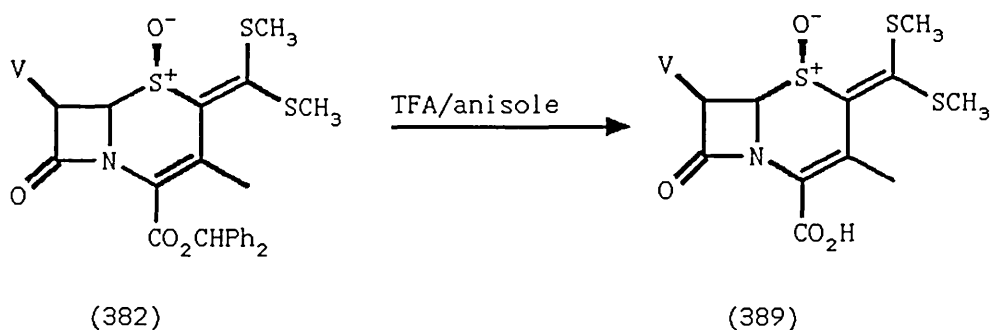
de-esterify (387) to obtain a sample of the acid for biological evaluation were unsuccessful. When the sulphide was stirred with zinc and acetic acid in DMF, a strong smell of methanethiol was noted. TLC indicated that reaction had occurred to give very polar components which could not be separated by chromatography. Yoshida and co-workers have reported that treatment of 2-methylthiolated cephem (388) with zinc/acetic acid results in loss of the methylthio group to afford (283)¹⁵⁹.



Thus, it is likely that zinc/acetic acid treatment of (387) results in removal of the trichloroethyl ester and the C-2 substituent. When diphenylmethyl ester (382) was treated with zinc in acetic acid/DMF, a strong smell of methanethiol was once again detected and after several hours, the starting material had been converted into a less polar component along with baseline

material. Chromatography of the crude product afforded a white powder in 18% yield which was identical (tlc,mp,pmr) to an authentic sample of sulfoxide (266).

Direct de-esterification of sulfoxide (382) with TFA/anisole resulted in a very polar product, the mass spectrum of which was consistent with loss of the diphenylmethyl protecting group. The pmr spectrum also indicated loss of the ester and all other signals were as expected, however no downfield signal could be detected for the C-4 carboxylic acid. An analytically pure sample of (389) could not be prepared, however the data obtained are in agreement with the proposed structure.



Acid (389) was not found to exhibit any antibacterial activity.

EXPERIMENTAL

EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer 1600 FT-IR or Perkin-Elmer 457 grating spectrophotometer, and ultraviolet absorptions were recorded as ethanolic solutions on either a Beckman DU-68 or Shimadzu UV-160 spectrophotometer. PMR spectra were recorded in deuteriochloroform (except where otherwise stated) on a Bruker WM250 instrument at 250 MHz or a Bruker WM400 at 400 MHz using tetramethylsilane as internal reference. Mass spectra were measured on either a VG 7070 or VG ZAB spectrometer operating in the electron impact mode. Fast atom bombardment spectra were recorded on a VG ZAB instrument using an appropriate matrix. Melting points were determined using an Electrothermal melting point apparatus and are uncorrected. Column chromatography was performed using pressurised short path columns with Kieselgel 60, particle size < 0.063mm (Merck # 7729). Reactions were monitored by thin layer chromatography on Merck DC-Alufohlen Kieselgel 60 F₂₅₄ (Merck # 5554) plates which were visualised under ultraviolet irradiation or with iodine vapour.

Penicillin V was kindly gifted by Beecham Pharmaceutical Research Division.

Diphenylmethyl (6R,7R) 3-Methyl-4 β -(3'-oxobutyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate (174).

The ester (153) (1.08g, 2mmol) in methyl vinyl ketone (15ml) was stirred with triethylamine (0.4ml) for 24h at

room temperature. The reaction mixture was partitioned between ethyl acetate and 1M HCl and successively washed with saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo*. Chromatography afforded diphenylmethyl (6R,7R) 3-methyl-4β-(3'-oxobutyl)-7β-phenoxyacetamidoceph-2-em-4-carboxylate (174) as a white crystalline solid (1.05g, 90%). mp 108-110°C. λ_{\max} 257nm (ϵ 6050). ν_{\max} (KBr) 3348, 1770, 1742, 1710, 1690 cm⁻¹. δ (250 MHz) 1.75 (3H,d,J=1.2Hz,3-CH₃), 2.11 (3H,s,COCH₃), 2.37-2.93 (4H,m,CH₂CH₂), 4.56 (2H,s,CH₂CON), 5.22 (1H,d,J=4.5Hz,6-H), 5.49 (1H,dd,J=4.5 & 8.8Hz,7-H), 6.11 (1H,d,J=1.2Hz,2-H), 6.88-7.55 (17H,m,PhO,CHPh₂ & NH). (Found : C,67.81; H,5.48; N,4.79; S,5.48. C₃₃H₃₂N₂O₆S requires C,67.42; H,5.47; N,4.79; S,5.58%). m/e 585 MH⁺.

Trichloroethyl (6R,7R) 3-Methyl-4β-(3'-oxobutyl)-7β-phenoxyacetamidoceph-2-em-4-carboxylate (237).

A solution of ester (168) (0.48g, 1mmol) in methyl vinyl ketone (10ml) containing triethylamine (0.2ml) was stirred for 18h at room temperature. Removal of solvents under reduced pressure afforded a brown semi-solid which was chromatographed on silica gel to yield trichloroethyl (6R,7R) 3-methyl-4β-(3'-oxobutyl)-7β-phenoxyacetamidoceph-2-em-4-carboxylate (237) (0.26g, 51%) as a white crystalline solid. mp 161-163°C. λ_{\max} 259nm (ϵ 5680). ν_{\max} (KBr) 3310, 1762, 1748, 1709, 1691cm⁻¹. δ (250MHz) 1.85 (3H,d,J=1.3Hz,3-CH₃), 2.18

(3H,s,COCH₃), 2.35-2.95 (4H,m,CH₂CH₂), 4.58 (2H,s,CH₂CON), 4.76 & 4.87 (2H,ABq,J=12.0Hz,CH₂CCl₃), 5.35 (1H,d,J=4.5Hz,6-H), 5.58 (1H,dd,J=4.5 & 8.8Hz,7-H), 6.20 (1H,d,J=1.3Hz,2-H), 6.92-7.40 (6H,m,PhO & NH). (Found : C,48.15; H,4.07; N,4.99; S,5.82. C₂₂H₂₃N₂Cl₃O₆S requires C,48.04; H,4.19; N,5.10; S,5.82%). m/e 548.0345 C₂₂H₂₃N₂Cl₃O₆S requires 548.0342.

Diphenylmethyl (6R,7R) 3-Methyl-4β-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-2-em-4-carboxylate (238).

Ester (153) (3.0g, 5.84mmol) in acrylonitrile (30ml) was stirred with triethylamine (0.5ml) at room temperature for 20h. Solvents were removed under reduced pressure and the residue partitioned between ethyl acetate and 1M HCl. The organic phase was washed with saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo*. The resulting oil was chromatographed to afford diphenylmethyl (6R,7R) 3-methyl-4β-(2'-cyanoethyl)-7β-phenoxyacetamidoceph-2-em-4-carboxylate (238) as a colourless oil (2.45g, 74%). λ_{max} 250nm (ε 9052). ν_{max} (film) 3345, 2248, 1773, 1738, 1690cm⁻¹. δ (250MHz) 1.67 (3H,d,J=1.2Hz,3-CH₃), 2.36-2.59 (3H,m,CH₂CHCN), 3.14-3.27 (1H,m,CHCN), 4.55 (2H,s,CH₂CON), 5.21 (1H,d,J=4.4Hz,6-H), 5.48 (1H,dd,J=4.4 & 8.8Hz,7-H), 6.31 (1H,d,J=1.2Hz,2-H), 6.88-7.42 (16H,m,PhO & CHPh₂), 7.53 (1H,d,J=8.8Hz,NH). (Found : C,67.57; H,5.05; N,7.30; S,5.46. C₃₂H₂₉N₃O₅S requires C,67.72; H,5.11; N,7.41; S,5.64%). m/e 568 MH⁺.

Diphenylmethyl (6R,7R) 3-Methyl-4 β -(1',2'-bis(methoxy-carbonyl)vinyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate (239).

A solution of ester (153) (5.14g, 10mmol) in acetonitrile was stirred at -15°C with dimethyl acetylendicarboxylate (3.12g, 22mmol) and triethylamine (1ml) for 1h. After warming to room temperature, solvents were removed *in vacuo* and the residue partitioned between ethyl acetate and 1M HCl. The organic phase was washed with saturated aqueous NaHCO₃ solution, brine, dried and solvent evaporated under reduced pressure. Chromatography afforded diphenylmethyl (6R,7R) 3-methyl-4 β -(1',2'-bis(methoxy-carbonyl)vinyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate (239) as a white crystalline solid (2.6g, 40%). mp 123-125°C. λ_{max} 254nm (ϵ 7903). ν_{max} (KBr) 3350, 1775, 1715, 1670 cm⁻¹. δ (250 MHz) 1.73 (3H,d,J=1.0Hz,3-CH₃), 3.39 (3H,s,CO₂CH₃), 3.83 (3H,s,CO₂CH₃), 4.54 (2H,s,CH₂CON), 5.42 (1H,d,J=4.4Hz,6-H), 5.63 (1H,dd,J=4.4 & 9.1Hz,7-H), 6.33 (1H,d,J=1.0Hz,2-H), 6.35 (1H,s,CHCO₂CH₃), 6.85-7.50 (17H,m,PhO,CHPh₂ & NH). (Found : C,63.90; H,4.92; N,4.33; S,4.83. C₃₅H₃₂N₂O₉S requires C,64.01; H,4.91; N,4.27; S,4.88%). m/e 657 MH⁺.

Attempted Reaction of (153) with Methyl Acrylate and Triethylamine.

The cephem (153) (0.514g, 1mmol) in methyl acrylate (10ml) containing triethylamine (0.2ml) was stirred at room temperature for 24h. TLC indicated formation of a slightly less polar material in addition to unreacted starting material. Chromatographic separation of the mixture after work-up afforded (153) and its Δ -2 isomer (335) in quantitative yield, identical to authentic samples.

Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7 β -phenoxy-acetamidoceph-3-em-4-carboxylate (153) with Methyl Acrylate.

The ester (153) (5.0g, 9.73 mmol) was stirred for 24h in methyl acrylate (40ml) containing Triton B (0.2ml, 40% w/v solution in methanol) at room temperature. The reaction mixture was diluted with ethyl acetate and washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo* to give a yellow oil. The R_f value of the starting material and product were identical on tlc, hence the oil was dissolved in dichloromethane (50ml) and stirred at 0°C with *m*-CPBA (2.0g, 80% pure hence 10mmol) for 30 minutes. The reaction mixture was filtered and the filtrate washed with 10% w/v aqueous Na₂S₂O₅ solution, saturated aqueous NaHCO₃ solution, brine, dried and

solvent removed under reduced pressure. Column chromatography afforded diphenylmethyl (1S,6R,7R) 3-methyl-4 β -(2'-(methoxycarbonyl)ethyl)-7 β -phenoxy-acetamidoceph-2-em-4-carboxylate 1-oxide (240) (3.17g, 53%) as a pale yellow solid after recrystallisation from ethyl acetate/petrol. mp 154-157°C. λ_{max} 256nm (ϵ 3200). ν_{max} (KBr) 3372, 1779, 1738, 1690cm⁻¹. δ (250 MHz) 1.81 (3H,d,J=1.2Hz,3-CH₃), 2.23-3.31 (4H,m,CH₂CH₂), 3.66 (3H,s,CO₂CH₃), 4.54 (3H,m,CH₂CON & H-6), 5.85 (1H,dd,J=4.7 & 10.5Hz,7-H), 6.77 (1H,d,J=1.2Hz,2-H), 6.91-7.43 (16H,m,PhO & CHPh₂), 8.17 (1H,d,J=10.5Hz,NH). (Found : C,64.34; H,5.21; N,4.25; S,4.85. C₃₃H₃₂N₂O₈S requires C,64.28; H,5.19; N,4.55; S,5.19%). m/e 617 MH⁺.

Also isolated was diphenylmethyl (1S,6R,7R) 3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (266) as a white crystalline solid (0.25g, 5%) which was identical to an authentic sample.

Methyl (6R,7R) 3-Methyl-4 β -(3'-oxobutyl)-7 β -phenoxy-acetamidoceph-2-em-4-carboxylate (244).

A solution of ceph-3-em (72) (0.54g, 1.5mmol) in methyl vinyl ketone (10ml) was stirred at room temperature with triethylamine (0.1ml) for 16h. The reaction mixture was partitioned between ethyl acetate and 1M HCl, and the organic layer washed with saturated aqueous NaHCO₃ solution, brine, dried and solvents removed *in vacuo*. Column chromatography afforded methyl (6R,7R) 3-methyl-4 β -(3'-oxobutyl)-7 β -phenoxyacetamido-

ceph-2-em-4-carboxylate (244) as a colourless oil (0.48g, 74%). λ_{max} 256nm (ϵ 4320). ν_{max} (film) 3320, 1768, 1742, 1711, 1693 cm^{-1} . δ (400 MHz) 1.80 (3H,d,J=1.2Hz, 3-CH₃), 2.17 (3H,s,COCH₃), 2.37-2.82 (4H,m, CH₂CH₂), 3.78 (3H,s,CO₂CH₃), 4.57 (2H,s,CH₂CON), 5.27 (1H,d,J=4.4Hz, 6-H), 5.58 (1H,dd,J=4.4 & 8.8Hz,7-H), 6.12 (1H,d,J= 1.2Hz,2-H), 6.95-7.36 (6H,m,PhO & NH). (Found : C,58.21; H,5.39; N,6.44; S,7.20. C₂₁H₂₄N₂O₆S requires C,58.33; H,5.55; N,6.48; S,7.41%). m/e 433 MH⁺.

Oxidation of (174) with m-CPBA.

Sulphide (174) (2.08g, 3.56mmol) was stirred in dichloromethane (60ml) with m-CPBA (0.77g, 80% pure hence 3.55mmol) at 0°C for 1h. The mixture was washed with 10% w/v aqueous Na₂S₂O₅ solution, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo*. Crystallisation from acetobne/petrol afforded diphenylmethyl (1S,6R,7R) 3-methyl-4 β -(3'-oxobutyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (38) as a white powder (1.66g, 85%). mp 175-177°C. λ_{max} 255nm (ϵ 6430). ν_{max} (KBr) 3300, 1770, 1730, 1700, 1680 cm^{-1} . δ (250 MHz) 1.80 (3H,d,J=0.9Hz,3-CH₃), 2.14 (3H,s,COCH₃), 2.49-3.10 (4H,m,CH₂CH₂), 4.52 (1H,d,J=4.8Hz,6-H), 4.53 (2H,s,CH₂CON), 5.84 (1H,dd,J=4.8 & 10.6Hz,7-H), 6.71 (1H,d,J=0.9Hz,2-H), 6.91-7.42 (16H,m,PhO & CHPh₂), 8.21 (1H,d,J=10.6Hz,NH). (Found : C,66.04; H,5.37; N,4.86; S,5.23. C₃₃H₃₂N₂O₇S requires C,66.00; H,5.33; N,4.66; S,5.33%). m/e 601 MH⁺.

Trichloroethyl (1S,6R,7R) 3-Methyl-4 β -(2'-cyanoethyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (245).

A solution of sulphide (234) (1.10g, 2.04mmol) in dichloromethane (80ml) at 0°C was stirred with *m*-CPBA (0.45g, 80% pure hence 2.1mmol) for 40 minutes. The reaction mixture was washed with 10% w/v aqueous Na₂S₂O₅ solution, saturated aqueous NaHCO₃ solution, brine, dried and solvent evaporated *in vacuo* to give a pale yellow solid. Recrystallisation from ethyl acetate/petrol gave trichloroethyl (1S,6R,7R) 3-methyl-4 β -(2'-cyanoethyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (245) as white crystals (0.50g, 44%). mp 147-148°C. λ_{max} 259nm (ϵ 3815). ν_{max} (KBr) 3320, 2247, 1778, 1755, 1688cm⁻¹. δ (400 MHz) 2.06 (3H,d,J=1.3Hz,3-CH₃), 2.58-2.78 (3H,m,CH₂CHCN), 3.31-3.41 (1H,m,CHCN), 4.54 & 4.58 (2H,ABq,J=15.2Hz,CH₂CON), 4.75 & 4.94 (2H,ABq,J=11.9Hz,CH₂CCl₃), 4.78 (1H,d,J=4.7Hz,6-H), 6.01 (1H,dd,J=4.7 & 10.5Hz,7-H), 6.94-7.34 (6H,m,PhO & 2-H), 8.09 (1H,d,J=10.5Hz,NH). (Found : C,45.94; H,3.64; N,7.62; S,5.61. C₂₁H₂₀N₃Cl₃O₈S requires C,45.94; H,3.65; N,7.66; S,5.83%). m/e 572 MNa⁺.

Oxidation of (236) with *m*-CPBA.

A solution of ceph-2-em (236) (2.40g, 3.86mmol) in dichloromethane (50ml) was stirred with *m*-CPBA (0.83g, 80% pure hence 3.9mmol) for 30 minutes. The reaction

mixture was washed with 10% w/v aqueous $\text{Na}_2\text{S}_2\text{O}_5$ solution, saturated aqueous NaHCO_3 solution, brine, dried and solvent removed under reduced pressure to afford, after chromatography, trichloroethyl (1S,6R,7R) 3-methyl-4 β -(1',2'-bis(methoxycarbonyl)vinyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (246) as a colourless oil. λ_{max} 261nm (ϵ 2500). ν_{max} (CHCl_3) 3378, 1789, 1761, 1727, 1690 cm^{-1} . δ (250 MHz) 2.14 (3H,d,J=1.3Hz,3- CH_3), 3.80 (3H,s, CO_2CH_3), 3.84 (3H,s, CO_2CH_3), 4.53 & 4.57 (2H,ABq,J=15.3Hz, CH_2CON), 4.74 & 4.92 (2H,ABq,J=11.9Hz, CH_2CCl_3), 4.87 (1H,d,J=4.7Hz,6-H), 6.04 (1H,dd,J=4.7 & 10.5Hz,7-H), 6.70 (1H,s, CHCO_2CH_3), 6.96 (1H,d,J=1.3Hz,2-H), 6.98-7.35 (5H,m,PhO), 8.14 (1H,d,J=10.5Hz,NH). (Found : M^+ 636.0140. $\text{C}_{24}\text{H}_{23}\text{N}_2\text{Cl}_3\text{O}_{10}\text{S}$ requires 636.0139).

Trichloroethyl (1S,6R,7R) 3-Methyl-4 β -(3'-oxobutyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (247).

The ester (237) (1.0g, 1.82mmol) in dichloromethane (40ml) was stirred at 0°C with *m*-CPBA (0.40g, 80% pure hence 1.85mmol) for 30 minutes. The reaction mixture was washed with 10% w/v aqueous $\text{Na}_2\text{S}_2\text{O}_5$ solution, saturated aqueous NaHCO_3 solution, brine, dried and concentrated *in vacuo*. Chromatography on silica gel afforded trichloroethyl (1S,6R,7R) 3-methyl-4 β -(3'-oxobutyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (247) as a colourless oil (0.86g, 85%). λ_{max} 254nm (ϵ 2037). ν_{max} (CHCl_3) 3373, 1780, 1705, 1690 cm^{-1} .

δ (250 MHz) 2.00 (3H,d,J=1.3Hz,3-CH₃), 2.17 (3H,s,COCH₃), 2.80-3.58 (4H,m,CH₂CH₂), 4.54 & 4.61 (2H, ABq,J=15.2Hz,CH₂CON), 4.74 & 4.92 (2H,ABq,J=9.8Hz, CH₂CCl₃), 4.77 (1H,d,J=4.7Hz,6-H), 6.00 (1H,dd,J=4.7 & 10.5Hz,7-H), 6.80 (1H,d,J=1.3Hz,2-H), 6.94-7.40 (5H,m, PhO), 8.23 (1H,d,J=10.5Hz,NH). (Found : M⁺ 564.0285. C₂₂H₂₅N₂Cl₃O₇S requires 564.0291).

Oxidation of (238) with *m*-CPBA.

Sulphide (238) (2.4g, 4.2mmol) dissolved in dichloromethane (60ml) was stirred with *m*-CPBA (0.9g, 80% pure hence 4.2mmol) at 0°C for 1h. The solution was washed with 10% w/v aqueous Na₂S₂O₅ solution, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo* to afford an off-white solid. Recrystallisation from ethyl acetate gave diphenylmethyl (1S,6R,7R) 3-methyl-4 β -(2'-cyanoethyl)-7 β -phenoxyacetamido-ceph-2-em-4-carboxylate 1-oxide (248) as white needles (2.2g, 90%). mp 160-162°C. λ_{max} 256nm (ϵ 2300). ν_{max} (KBr) 3370, 2244, 1781, 1740, 1684cm⁻¹. δ (250 MHz) 1.83 (3H,d,J=1.4Hz,3-CH₃), 2.49-2.79 (3H,m,CH₂CHCN), 3.29-3.41 (1H,m,CHCN), 4.55 (2H,s,CH₂CON), 4.59 (1H,d,J=4.6Hz,6-H), 5.87 (1H,dd,J=4.6 & 10.5Hz,7-H), 6.89 (1H,d,J=1.4Hz,2-H), 6.93-7.43 (16H,m,PhO & CHPh₂), 8.08 (1H,d,J=10.5Hz,NH). (Found : C,65.69; H,4.95; N,7.11; S,5.54. C₃₂H₂₉N₃O₆S requires C,65.87; H,4.97; N,7.20; S,5.49%). m/e 606 MNa⁺.

Reaction of (38) with Trifluoroacetic Acid and Anisole.

The ester (38) (0.85g, 1.42mmol) dissolved in anisole (1.2ml) and trifluoroacetic acid (3.6ml) was stirred at room temperature for 15 minutes. Solvents were removed *in vacuo* and the residue partitioned between ethyl acetate and 1M HCl. The organic phase was washed with brine, dried and solvent removed under reduced pressure to afford the unstable acid (252) (0.47g, 76%). mp (crude) 110°C (decomp.). ν_{max} (KBr) 3348, 3060-2580, 1778, 1752, 1710, 1682cm⁻¹. Attempted recrystallisation of (252) from refluxing acetone resulted in (1S,6R,7R) 3-methyl-4-(3'-oxobutyl)-7 β -phenoxyacetamidoceph-3-em 1-oxide (253) (0.40g, 72%) as a flocculent white solid. mp > 310°C. λ_{max} 251nm (ϵ 8300). ν_{max} (KBr) 3351, 1761, 1703, 1689cm⁻¹. δ (250 MHz) 1.85 (3H,s,3-CH₃), 2.18 (3H,s,COCH₃), 2.68-3.03 (4H,m,CH₂CH₂), 3.15 & 3.43 (2H,ABq,J=18.0Hz,2-H), 4.41 (1H,dd,J=4.6 & 1.2Hz,6-H), 4.58 (2H,s,CH₂CON), 6.01 (1H,dd,J=4.6 & 10.4Hz,7-H), 6.92-7.35 (5H,m,PhO), 7.96 (1H,d,J=10.4Hz,NH). (Found : C,58.87; H,5.59; N,7.08; S,8.17. C₁₉H₂₂N₂O₅S requires C,58.46; H,5.64; N,7.18; S,8.21%). m/e 390.1241 M⁺.

De-oxygenation of (253) with P₂S₅/Pyridine.

Sulphoxide (253) (0.20g, 0.51mmol) dissolved in dichloromethane containing pyridine (0.16g, 2.1mmol) was stirred with P₂S₅ (0.06g, 0.26mmol) for 12h. The reaction mixture was diluted with dichloromethane and washed with 1M HCl, H₂O, brine, dried and solvents

evaporated under reduced pressure. Column chromatography afforded (6R,7R) 3-methyl-4-(3'-oxobutyl)-7 β -phenoxyacetamidoceph-3-em (175) as an off-white powder (0.27g, 73%). mp 160-162°C. λ_{max} 259nm (ϵ 8900). ν_{max} (KBr) 3250, 1760, 1700, 1670cm⁻¹. δ (250 MHz) 1.80 (3H,s,3-CH₃), 2.17 (3H,s,COCH₃), 2.64-2.87 (4H,m,CH₂CH₂), 3.22 (2H,ABq,J=17.3Hz,2-H), 4.57 (2H,s,CH₂CON), 4.94 (1H,d,J=4.8Hz,6-H), 5.77 (1H,dd, J=4.8 & 10.5Hz,7-H), 6.92-7.36 (6H,m,PhO & NH). (Found : C,60.61; H,5.64; N,7.51; S,8.41. C₁₉H₂₂N₂O₄S requires C,60.96; H,5.88; N,7.49; S,8.56%). m/e 375 MH⁺.

Reaction of Trichloroethyl (1S,6R,7R) 3-Methyl-4-(3'-oxobutyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (247) with Zinc and Acetic Acid.

A solution of the ester (247) (0.85g, 1.5mmol) in DMF (10ml) and glacial acetic acid (3ml) was stirred with powdered zinc (1.3g) for 1h at room temperature. The reaction mixture was filtered, and the filtrate partitioned between saturated aqueous NaHCO₃ solution and ethyl acetate. The organic phase was washed with brine, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo* to afford a yellow semi-solid. Crystallisation from ethyl acetate afforded (6R,7R) 3-methyl-4-(3'-oxobutyl)-7 β -phenoxyacetamidoceph-3-em 1-oxide (253) as a flocculent white solid (0.25g, 29%), which was found to be identical to a sample prepared above.

De-esterification and Decarboxylation of Diphenylmethyl (1S,6R,7R) 3-Methyl-4 β -(3'-oxobutyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (38).

The ester (38) (0.60g, 1mmol) in anisole (1.0ml) and trifluoroacetic acid (3.0ml) was stirred at room temperature for 15 minutes before solvents were removed *in vacuo*. The crude acid was dissolved in ethyl acetate containing triethylamine (0.5ml) and stirred at room temperature for 12h. The reaction mixture was partitioned between ethyl acetate and 1M HCl and the organic phase washed with saturated aqueous NaHCO₃ solution, brine, dried and solvent removed under reduced pressure. The resulting oil was chromatographed to afford (253) (0.31g, 79%) as a flocculent white solid, identical in all respects to a sample prepared above.

Attempted De-esterification and Decarboxylation of Diphenylmethyl (6R,7R) 3-Methyl-4 β -(3'-oxobutyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate (174).

A solution of sulphide (174) (0.85g, 1.45mmol) in anisole (1.2ml) and trifluoroacetic acid (3.6ml) was stirred at room temperature for 10 minutes. Solvents were removed *in vacuo* and the residue partitioned between ethyl acetate and dilute aqueous NaHCO₃ solution. The aqueous portion was acidified with 1M HCl and extracted with fresh ethyl acetate. After washing with brine and drying, the solvent was evaporated under

reduced pressure to yield a white foam. Crystallisation from ethyl acetate/dichloromethane gave (6R,7R) 3-methyl-4-(3'-oxobutyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylic acid (254) as a white crystalline solid (0.52g, 87%). mp 170-171°C. λ_{max} 254nm (ϵ 5093), 274nm (ϵ 1786). ν_{max} (KBr) 3700-3000, 1760, 1720, 1680cm⁻¹. δ (d₆-DMSO, 250 MHz) 1.70 (3H,d,J=1.2Hz,3-CH₃), 2.10 (3H,s,COCH₃), 2.18-2.72 (4H,m,CH₂CH₂), 4.51 & 4.62 (2H,ABq,J=15.0Hz,CH₂CON), 5.10 (1H,d,J=4.3Hz,6-H), 5.29 (1H,dd,J=4.3 & 7.6Hz,7-H), 6.38 (1H,d,J=1.2Hz,2-H), 6.92-7.35 (5H,m,PhO), 9.12 (1H,d,J=7.6Hz,NH). (Found : C,57.30; H,5.26; N,6.52; S,7.60. C₂₀H₂₂N₂O₆S requires C,57.42; H,5.26; N,6.70; S,7.66%). m/e 441 MNa⁺.

Attempted Decarboxylation of (254) with Triethylamine.

A solution of acid (254) (0.25g, 0.6mmol) in dichloromethane (10ml) containing triethylamine (0.1ml) was stirred at room temperature for 4 days. TLC analysis indicated no reaction of (254), and the reaction mixture was partitioned between dichloromethane and 1M HCl. The organic portion was washed with brine, dried and solvent removed *in vacuo* resulting in quantitative recovery of (254).

(1S,6R,7R) 3-Methyl-4-(2'-cyanoethyl)-7 β -phenoxy-acetamidoceph-3-em 1-oxide (255).

A solution of ester (248) (1.17g, 2mmol) in dichloromethane (30ml) was stirred with anisole (0.5ml)

and trifluoroacetic acid (2.5ml) at 0°C for 30 minutes. Solvents were removed *in vacuo* and the residue stirred in acetone containing triethylamine (0.3ml) for 8h. The reaction mixture was filtered to yield crude **(255)** which was recrystallised from refluxing acetone to afford (1S,6R,7R) 3-methyl-4-(2'-cyanoethyl)-7β-phenoxy-acetamidoceph-3-em 1-oxide (255) (0.58g, 78%) as a white crystalline solid. mp 206-208°C. λ_{max} 250nm (ϵ 9052). ν_{max} (KBr) 3345, 2246, 1765, 1685cm⁻¹. δ (CDCl₃ + CD₃OD, 250 MHz) 1.91 (3H,s,3-CH₃), 2.68-2.98 (3H,m,CH₂CHCN), 3.17-3.25 (1H,m,CHCN), 3.31 & 3.52 (2H,ABq,J=18.3Hz, 2-H), 4.57 (1H,d,J=4.5Hz,6-H), 4.59 (2H,s,CH₂CON), 6.02 (1H,d,J=4.5Hz,7-H), 6.93-7.37 (5H,m,PhO), NH exchanged with CD₃OD. (Found : C,57.85; H,5.30; N,11.30; S,8.27. C₁₈H₁₉N₃O₄S requires C,57.91; H,5.09; N,11.26; S,8.58%). m/e 373.1086 M⁺.

(1S,6R,7R) 3-Methyl-4-(2'-(methoxycarbonyl)ethyl)-7β-phenoxyacetamidoceph-3-em 1-oxide (256).

Ester **(240)** (0.15g, 0.24mmol) was stirred in anisole (1.3ml) and trifluoroacetic acid (3ml) for 10 minutes. Solvents were removed under reduced pressure and the residue partitioned between ethyl acetate and dilute aqueous NaHCO₃ solution. The aqueous portion was acidified with 1M HCl and extracted with ethyl acetate. The organic phase was then washed with brine, dried and solvent evaporated *in vacuo*. The residual foam was dissolved in ethyl acetate and stirred with

triethylamine (0.1ml) for 5h. The reaction mixture was filtered to yield a crude solid which was recrystallised from refluxing acetone to afford (1S,6R,7R) 3-methyl-4-(2'-(methoxycarbonyl)ethyl)-7 β -phenoxyacetamido-ceph-3-em 1-oxide (256) as a white powder (0.08g, 82%). mp 162-164°C. λ_{max} 250nm (ϵ 7135). ν_{max} (KBr) 3351, 1762, 1728, 1686cm⁻¹. δ (250 MHz) 1.86 (3H,s,3-CH₃), 2.62-2.98 (4H,m,CH₂CH₂), 3.16 & 3.44 (2H,ABq,J=17.8Hz, 2-H), 3.68 (3H,s,CO₂CH₃), 4.42 (1H,d,J=4.6Hz,6-H), 4.58 (2H,s,CH₂CON), 6.02 (1H,dd,J=4.6 & 10.4Hz,7-H), 6.93-7.34 (5H,m,PhO), 7.96 (1H,d,J=10.4Hz,NH). (Found : C,56.01; H,5.24; N,6.84; S,7.80. C₁₉H₂₂N₂O₆S requires C,56.16; H,5.42; N,6.89; S,7.88%). m/e 406.1198, C₁₉H₂₂N₂O₆S requires 406.1199.

De-oxygenation of (256).

A solution of sulfoxide (256) (0.33g, 0.8mmol) in dichloromethane (25ml) containing pyridine (0.25g, 3.2mmol) was stirred with P₂S₅ (0.09g, 0.4mmol) for 2h. The reaction mixture was partitioned between water and dichloromethane and the organic portion washed with 1M HCl, brine, dried and solvent removed *in vacuo* to give a pale green semi-solid. Recrystallisation from petrol/dichloromethane afforded (6R,7R) 3-methyl-4-(2'-(methoxycarbonyl)ethyl)-7 β -phenoxyacetamidoceph-3-em (257) as a white crystalline solid (0.19g, 60%). mp 147-148°C. λ_{max} 268nm (ϵ 4000) 326nm (ϵ 11050). ν_{max} (KBr) 3287, 1791, 1727, 1691cm⁻¹. δ (250 MHz) 1.80 (3H,s,3-CH₃),

2.55-2.87 (4H,m,CH₂CH₂), 2.98 & 3.47 (2H,ABq,J=17.3Hz, 2-H), 3.68 (3H,s,CO₂CH₃), 4.56 (2H,s,CH₂CON), 4.95 (1H,d,J=4.8Hz,6-H), 5.77 (1H,dd,J=4.8 & 9.2Hz,7-H), 6.90-7.36 (6H,m,PhO & NH). (Found : C,58.53; H,5.37; N,7.04; S,8.29. C₁₉H₂₂N₂O₅S requires C,58.46; H,5.64; N,7.18; S,8.20%). m/e 414 MNa⁺.

Attempted Reaction of (249) with Trifluoroacetic Acid/Anisole.

A solution of the triester (249) (1.0g, 1.5mmol) in dichloromethane (40ml) was stirred with anisole (2.43g) and trifluoroacetic acid (2.56g) at 0°C for 2h. TLC indicated no starting material remained and solvents were removed *in vacuo* to afford a brown oil. The oil was dissolved in dichloromethane and stirred with triethylamine (0.4ml) at room temperature for 20h. The solution was diluted with dichloromethane and washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed under reduced pressure to give a brown oil. Column chromatography appeared to afford a major component which was found to be highly complex by pmr spectroscopy. Repeated column chromatography and preparative tlc both failed to result in any pure material and the reaction was not examined further.

Reaction of (239) with Trifluoroacetic Acid/Anisole.

Ceph-2-em (239) (0.5g, 0.76mmol) was reacted with trifluoroacetic acid (1.76ml) and anisole (2.48ml) at

room temperature for 45 minutes. Solvents were removed *in vacuo* and the resulting yellow oil dissolved in dichloromethane containing triethylamine (0.1ml) and stirred for 1h. The solution was washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvents evaporated under reduced pressure to afford a white solid. Recrystallisation from ethyl acetate/petrol yielded (6R,7R) 3-methyl-4-(1',2'-bis(methoxycarbonyl)vinyl)-7β-phenoxyacetamidoceph-3-em (260) (0.25g, 84%). mp 125-126°C. λ_{max} 259nm (ϵ 9800). ν_{max} 3278, 1766, 1731, 1668cm⁻¹. δ (250 MHz) 1.95 (3H,s, 3-CH₃), 3.32 & 3.77 (2H,ABq,J=17.5Hz,2-H), 3.72 (3H,s,CO₂CH₃), 3.80 (3H,s,CO₂CH₃), 4.56 (2H,s,CH₂CON), 5.20 (1H,d,J=4.5Hz,6-H), 5.81 (1H,dd,J=4.5 & 9.0Hz, 7-H), 6.32 (1H,s,CHCO₂CH₃), 6.90-7.37 (6H,m,PhO & NH). (Found : C,56.57; H,4.83; N,6.20; S,7.07. C₂₁H₂₂N₂O₇S requires C,56.50; H,4.93; N,6.28; S,7.17%). m/e 447 MH⁺.

(6R,7R) 3-Methyl-4-(1',2'-bis(methoxycarbonyl)ethyl)-7β-phenoxyacetamidoceph-3-em 1-oxide (262).

Triester (246) (0.8g, 1.25mmol) dissolved in glacial acetic acid (5ml) and DMF (12ml) was stirred at room temperature with zinc powder (1.6g) for 2.5h. The reaction mixture was filtered, and the filtrate partitioned between ethyl acetate and brine. The organic phase was washed with saturated aqueous NaHCO₃ solution, brine, dried and evaporated to give a colourless oil. Crystallisation from dichloromethane/

petrol gave a flocculent white precipitate of (6R,7R)-3-methyl-4-(1',2'-bis(methoxycarbonyl)ethyl)-7β-phenoxyacetamidoceph-3-em 1-oxide (262) (0.22g, 38%). mp 169-170°C. λ_{max} 251nm (ϵ 8850). ν_{max} (KBr) 3450-3200, 1771, 1728, 1693cm⁻¹. δ (250 MHz) 1.91 (3H,s,3-CH₃), 2.78 (1H,dd,J=17.1 & 7.5Hz,CH₂CO₂CH₃), 3.20 & 3.51 (2H,ABq,J=18.0Hz,2-H), 3.25 (1H,dd,J=17.1 & 7.2Hz,CH₂CO₂CH₃), 3.71 (3H,s,CO₂CH₃), 3.72 (3H,s,CO₂CH₃), 4.36 (1H,dd,J=7.5 & 7.2Hz,CHCO₂CH₃), 4.42 (1H,dd,J=1.2 & 4.7Hz,6-H), 4.57 (2H,s,CH₂CON), 6.01 (1H,dd,J=4.7 & 10.4Hz,7-H), 6.95-7.30 (5H,m,PhO), 7.94 (1H,d,J=10.4Hz,NH). (Found : C,54.13; H,4.83; N,5.91; S,6.60. C₂₁H₂₄N₂O₈S requires C,54.31; H,5.17; N,6.03; S,6.90%). m/e 464 M⁺

Attempted De-oxygenation of (262).

Sulphoxide (262) (0.5g, 1.08mmol) in dichloromethane (20ml) was stirred with pyridine (0.34g, 4.3mmol) and P₂S₅ (0.12g, 0.54mmol) for 2h. The reaction mixture was diluted with dichloromethane and washed with 1M HCl, H₂O, brine, dried and solvent removed *in vacuo* to give a yellow oil. TLC analysis indicated a complex mixture which could not be separated by column chromatography or preparative tlc.

Reaction of (239) with Zinc/Acetic Acid.

A solution of ester (239) (1.0g, 1.5mmol) in DMF (12ml) and glacial acetic acid (4ml) was stirred with

powdered zinc (1.5g) at room temperature for 75 minutes. Zinc was removed by filtration and the filtrate partitioned between ethyl acetate and brine. The organic phase was washed with saturated aqueous NaHCO₃ solution, brine, dried and solvent evaporated *in vacuo* to give a white foam. Crystallisation from dichloromethane/ether afforded diphenylmethyl (6R,7R) 3-methyl-4-(1',2'-bis(methoxycarbonyl)ethyl)-7β-phenoxyacetamidoceph-2-em-4-carboxylate (263) as a white crystalline solid (0.45g, 46%). mp 89-97°C. λ_{max} 257nm (ϵ 2180). ν_{max} (KBr) 3413, 1773, 1740, 1692cm⁻¹. δ (250 MHz) 1.78 (3H,d,J=1.0Hz,3-CH₃), 2.25 (1H,dd,J=1.6 & 17.0Hz,CH₂CO₂CH₃), 3.02 (1H,dd,J=9.4 & 17.0Hz, CH₂CO₂CH₃), 3.45 (3H,s,CO₂CH₃), 3.75 (3H,s,CO₂CH₃), 4.38 (1H,dd,J=1.6 & 9.4Hz,CHCO₂CH₃), 4.54 (2H,s,CH₂CON), 5.51 (1H,d,J=4.6Hz,6-H), 5.57 (1H,dd,J=4.6 & 9.1Hz,7-H), 6.20 (1H,d,J=1.0Hz,2-H), 6.90-7.42 (17H,m,PhO,CHPh₂ & NH). (Found : M⁺ 658.1989. C₃₅H₃₄N₂O₉S requires 658.1986).

Reaction of Diphenylmethyl (1S,6R,7R) 3-Methyl-4β-(1',2'-bis(methoxycarbonyl)vinyl)-7β-phenoxyacetamidoceph-2-em-4-carboxylate (249) with Zinc and Acetic Acid.

A solution of sulfoxide (249) (0.2g, 0.3mmol) in DMF and glacial acetic acid (10ml, 25:7.5 v/v) was stirred at room temperature with powdered zinc (1.5g) for 5h. Zinc was removed by filtration and the filtrate diluted with ethyl acetate. After washing with saturated aqueous NaHCO₃ solution, brine and drying, removal of

solvents under reduced pressure afforded (263) as a white crystalline solid (0.15g, 75%) which was identical in all respects to an authentic sample.

Reaction of (248) with Zinc and Acetic Acid.

A solution of sulfoxide (248) (0.22g, 0.38mmol) in DMF/glacial acetic acid (10ml, 25:7.5 v/v) was stirred with zinc powder (1.0g) at room temperature for 6h. The reaction mixture was filtered and the filtrate diluted with ethyl acetate and washed with brine, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo*. Column chromatography afforded (238) as a colourless oil (0.21g, 97%) which was found to be identical in all respects to an authentic sample.

Diphenylmethyl (6R,7R) 3-Methyl-4 β -(2'-(methoxycarbonyl)ethyl-7 β -phenoxyacetamidoceph-2-em-4-carboxylate (265).

Sulfoxide (240) (0.18g, 0.3mmol) in DMF/glacial acetic acid (10ml, 25:7.5 v/v) was stirred with powdered zinc (1.5g) at room temperature for 6h. Removal of zinc by filtration followed by dilution with ethyl acetate and washing the organic portion with saturated aqueous NaHCO₃ solution, brine, drying and removal of solvents *in vacuo* gave diphenylmethyl (6R,7R) 3-methyl-4 β -2'-(methoxycarbonyl)ethyl-7 β -phenoxyacetamido-ceph-2-em-4-carboxylate (265) as a colourless oil (0.14g, 80%). λ_{max} 261nm (ϵ 4810). ν_{max} 3312, 1773, 1736, 1686cm⁻¹. δ (250 MHz) 1.72 (3H,d, J=0.9Hz,3-CH₃),

2.18-3.08 (4H,m,CH₂CH₂), 3.67 (3H,s,CO₂CH₃), 4.56 (2H,s,CH₂CON), 5.19 (1H,d,J=4.4Hz,6-H), 5.49 (1H,dd, J=4.4 & 8.9Hz,7-H), 6.17 (1H,d,J=0.9Hz,2-H), 6.88-7.41 (17H,m,PhO,CHPh₂ & NH). (Found : C,65.76; H,5.40; N,4.49; S,5.25. C₃₃H₃₂N₂O₇S requires C,66.00; H,5.33; N,4.66; S,5.33%). m/e 623 MNa⁺.

Attempted De-oxygenation of Sulphoxide (266).

Sulphoxide (266) (0.20g, 0.38mmol) in DMF and glacial acetic acid (10ml, 25:7.5 v/v) was stirred with zinc powder (1.0g) at room temperature. After 72h, no trace of reaction was observed and after work-up, (266) was isolated in quantitative yield.

Attempted Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate (153) with Trichloroethyl Acrylate.

A solution of ester (153) (0.26g, 0.5mmol) in trichloroethyl acrylate (5ml) was stirred at room temperature with Triton B (0.1ml, 40% w/v solution in methanol) for 17h. The solution was diluted with ethyl acetate and washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and concentrated *in vacuo* to give a white solid. Chromatography on silica gel afforded firstly diphenylmethyl (6R,7R) 3-methyl-7β-phenoxy-acetamidoceph-2-em-4-carboxylate (335) as a white powder (0.75g, 36%), identical to an authentic sample.

Further elution gave diphenylmethyl (6R,7R) 3-methyl-

7 β -phenoxyacetamidoceph-3-em-4-carboxylate (153) as an amorphous white solid (1.20g, 58%).

Attempted Reaction of (153) with *p*-Nitrobenzyl Acrylate.

A mixture of cephem (153) (0.1g, 0.2mmol) and *p*-nitrobenzyl acrylate (1.0g) was warmed to 60°C and Triton B (0.05ml, 40% w/v solution in methanol) was added. The reaction mixture was maintained at 60°C for 96h. TLC analysis of the reaction mixture indicated an equilibrium mixture of Δ -2 and Δ -3 isomers (335) and (153). Two further components were found to have R_f values identical to *p*-nitrobenzyl alcohol and acrylic acid, and the reaction was not further investigated.

Attempted Reaction between (153) and Acrolein.

A solution of cephem (153) (0.514g, 1mmol) in acrolein (20ml) was stirred with triethylamine (0.1ml) at room temperature. After 15 minutes, the reaction mixture had become completely solid and the glassy yellow product could not be dissolved in a variety of solvents. Further examination of the reaction was not carried out.

Reaction of (256) with Sodium Hydroxide.

Sulphoxide (256) (0.20g, 0.5mmol) was stirred in THF (20ml) and DMF (2ml) with aqueous sodium hydroxide solution (5ml, 0.1M) at ice temperature for 4h. TLC indicated apparent reaction and the solution was partitioned between ethyl acetate and 1M HCl. The

organic layer was washed with brine, dried and solvent removed *in vacuo* to give a dark yellow oil. The infrared spectrum of this oil indicated loss of the β -lactam carbonyl stretching frequency, and the reaction was not further investigated.

(1S,6R,7R) 3-Methyl-4-(2'-carboxyethyl)-7 β -phenoxy-acetamidoceph-3-em 1-oxide (271).

Cephem (256) (0.2g, 0.5mmol) was refluxed in toluene (8ml) containing bis(tri-*n*-butyl)tin oxide (0.5ml) and 2,2-azobis(2-methylpropionitrile) (*ca.* 1mg) for 2h. After cooling to room temperature, the reaction mixture was partitioned between ethyl acetate and saturated aqueous NaHCO₃ solution. The aqueous portion was acidified with 1M HCl and extracted with fresh ethyl acetate. This organic phase was washed with brine, dried and solvent removed *in vacuo* to give an off-white solid. Attempted recrystallisation from ether afforded (1S,6R,7R) 3-Methyl-4-(2'-carboxyethyl)-7 β -phenoxy-acetamidoceph-3-em 1-oxide (271). (0.03g, 15%). mp 132°C (decomp.). ν_{max} (KBr) 3356, 3280-2300, 1770, 1697cm⁻¹. δ (d₆-DMSO, 400 MHz) 1.75 (3H,s,3-CH₃), 2.44-2.86 (4H,m,CH₂CH₂), 3.46 (2H,s,2-H), 4.67 (2H,s,CH₂CON), 4.79 (1H,d,J=4.6Hz, 6-H), 5.84 (1H,dd,J=4.6 & 9.8Hz,7-H), 6.94-7.35 (5H,m, PhO), 8.18 (1H,d,J=9.8Hz,NH), 12.24 (1H,bs,CO₂H). m/e 393 MH⁺.

Diphenylmethyl (6R,7R) 3-Methyl-4 β -(2'-cyanoethyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1,1-dioxide (272).

A solution of sulphoxide (248) (0.08g, 0.14mmol) in dichloromethane (10ml) was stirred with *m*-CPBA (0.08g, 80% pure hence 0.4mmol) for 24h. The reaction mixture was diluted with dichloromethane and washed with 10% w/v aqueous Na₂S₂O₅ solution, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed under reduced pressure to give a white solid. Recrystallisation from ethyl acetate/petrol afforded diphenylmethyl (6R,7R) 3-methyl-4 β -(2'-cyanoethyl)-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1,1-dioxide (272) as an amorphous white solid (0.07g, 85%). mp 144-145°C. λ_{max} 268nm (ϵ 1526). ν_{max} (KBr) 3391, 2247, 1782, 1735, 1688cm⁻¹. δ (250 MHz) 1.80 (3H,d,J=1.4Hz,3-CH₃), 2.33 (1H,m,CH₂CH₂CN), 2.60 (2H,m,CH₂CH₂CN), 3.30 (1H,m,CH₂CH₂CN), 4.52 & 4.59 (2H,ABq,J=15.2Hz,CH₂CON), 4.80 (1H,d,J=4.7Hz,6-H), 5.93 (1H,dd,J=4.7 & 10.7Hz,7-H), 6.53 (1H,d,J=1.4Hz,2-H), 6.88-7.48 (16H,m,PhO & CHPh₂), 8.07 (1H,d,J=10.7Hz,NH). (Found : C,63.85; H,4.69; N,6.95; S,4.98. C₃₂H₂₉N₃O₇S requires C,64.11; H,4.48; N,7.01; S,5.34%). m/e 599.1716, C₃₂H₂₉N₃O₇S requires 599.1726.

(6R,7R) 3-Methyl-4-(2'-cyanoethyl)-7 β -phenoxyacetamido-ceph-3-em 1,1-dioxide (273).

A solution of ester (272) (0.3g, 0.5mmol) in dichloromethane (20ml) at 0°C was stirred with anisole (0.2ml) and trifluoroacetic acid (1.5ml) for 30 minutes. Solvents were removed *in vacuo* to give an oil which was redissolved in ethyl acetate and stirred for 15h with triethylamine (0.1ml). The reaction mixture was washed with 1M HCl, brine, dried and solvent removed under reduced pressure to afford a white solid.

Recrystallisation from dichloromethane/petrol afforded (6R,7R) 3-methyl-4-(2'-cyanoethyl)-7 β -phenoxyacetamido-ceph-3-em 1,1-dioxide (273) as white crystals (0.20g, 82%). mp 154-157°C. λ_{max} 245nm (ϵ 8715). ν_{max} (KBr) 3346, 2250, 1777, 1693cm⁻¹. δ (250 MHz) 1.85 (3H,s,3-CH₃), 2.69 (2H,m,CH₂CH₂CN), 2.77 (1H,m,CHCN), 3.07 (1H,m,CHCN), 3.45 & 3.90 (2H,ABq,J=18.0Hz,2-H), 4.57 & 4.61 (2H,ABq,J=15.4Hz,CH₂CON), 4.84 (1H,d,J=4.7Hz,6-H), 6.14 (1H,dd,J=4.7 & 10.6Hz,7-H), 6.92-7.29 (5H,m,PhO), 7.98 (1H,d,J=10.6Hz,NH). (Found : C,55.26; H,4.78; N,10.59; S,8.04. C₁₈H₁₇N₃O₅S requires C,55.53; H,4.88; N,10.80; S,8.23%). m/e 412 MNa⁺.

Diphenylmethyl (6R,7R) 2,2-bis(2'-Cyanoethyl)-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1,1-dioxide (275).

Sulphone (274) (0.55g, 1mmol) in acrylonitrile (20ml)

was stirred at room temperature with triethylamine (0.1ml) for 20h. The solution was diluted with ethyl acetate and washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent evaporated *in vacuo*. Column chromatography afforded diphenylmethyl (6R,7R) 2,2-bis(2'-cyanoethyl)-3-methyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylate 1,1-dioxide (275) (0.35g, 54%) as a white foam. λ_{max} 264nm (ϵ 1821). ν_{max} (KBr) 3403, 2250, 1790, 1744, 1702cm⁻¹. δ (400 MHz) 1.83 (3H,s,3-CH₃), 2.47-3.31 (8H,m,2 x CH₂CH₂CN), 4.53 (2H,s,CH₂CON), 4.76 (1H,d,J=4.6Hz,6-H), 5.90 (1H,dd,J=4.6 & 10.6Hz,7-H), 6.92-7.42 (16H,m,PhO & CHPh₂), 7.98 (1H,d,J=10.6Hz,NH). (Found : C,64.26; H,5.07; N,8.46; S,4.94. C₃₅H₃₂N₄O₇S requires C,64.32; H,4.90; N,8.58; S,4.90%). m/e 675 MNa⁺.

Reaction of Diphenylmethyl (1S,6R,7R) 3-Methyl-7β-phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (266) with NaH and Iodomethane.

A solution of sulphoxide (266) (2.06g, 3.9mmol) in DMF (25ml) was stirred at 0°C under an N₂ atmosphere with iodomethane (0.67g, 4.7mmol). To the reaction mixture was added sodium hydride (0.11g, 4.6mmol) and stirring continued at 0°C for 2h. The solution was poured onto ice/water, extracted with ethyl acetate and the organic portion washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo*. Column chromatography afforded firstly diphenylmethyl

(1S,6R,7R)-2,2,3-trimethyl-7 β -phenoxy-acetamidoceph-3-em-4-carboxylate 1-oxide (293) as a white powder (0.20g, 9%). mp 192-194°C. λ_{max} 264nm (ϵ 1896). ν_{max} (KBr) 3363, 1764, 1739, 1693cm⁻¹. δ (250 MHz) 1.64 (3H,d,J=0.9Hz,2-CH₃), 2.08 (3H,s,3-CH₃), 2.20 (3H,d,J=0.9Hz,2-CH₃), 4.54 (2H,s,CH₂CON), 4.60 (1H,d,J=4.5Hz,6-H), 5.85 (1H,dd,J=4.5 & 10.6Hz,7-H), 6.89-7.43 (16H,m,PhO & CHPh₂), 8.16 (1H,d,J=10.6Hz,NH). (Found : C,66.32; H,5.69; N,5.00; S,6.08. C₃₁H₃₀N₂O₆S requires C,66.66; H,5.37; N,5.02; S,5.73%). m/e 581 MNa⁺.

Further elution afforded diphenylmethyl (1S,6R,7R) 3,4-dimethyl-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (291) as a white foam (1.30g, 62%). λ_{max} 253nm (ϵ 2570). ν_{max} (KBr) 3354, 1784, 1733, 1690cm⁻¹. δ (250 MHz) 1.78 (3H,d,J=1.2Hz,3-CH₃), 2.01 (3H,s,4-CH₃), 4.58 (2H,s,CH₂CON), 4.73 (1H,d,J=4.5Hz,6-H), 6.01 (1H,dd,J=4.5 & 10.8Hz,7-H), 6.73 (1H,d,J=1.2Hz,2-H), 6.95-7.39 (16H,m,PhO & CHPh₂), 8.21 (1H,d,J=10.8Hz,NH). (Found : C,66.51; H,5.16; N,5.00; S,5.57. C₃₀H₂₈N₂O₆S requires C,66.17; H,5.15; N,5.15; S,5.88%). m/e 567 MNa⁺.

Diphenylmethyl (1S,6R,7R) 2-*t*-Butyloxycarbonylmethyl-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (298).

Sulphoxide (266) (0.53g, 1mmol) in DMF (25ml) was stirred at 0°C under an N₂ atmosphere with *t*-butyl bromoacetate (0.23g, 1.2mmol) and sodium hydride (0.03g, 1.25mmol) for 3h. The reaction mixture was poured onto

ice/water and extracted with ethyl acetate. The organic portion was washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent evaporated *in vacuo* to give a brown oil. Chromatography afforded as the only β -lactam containing material diphenylmethyl (1S,6R,7R) 2-*t*-butyloxycarbonylmethyl-3-methyl-7 β -phenoxy-acetamidoceph-3-em-4-carboxylate 1-oxide (298) as a white crystalline solid (0.41g, 64%). mp 149-151°C. λ_{max} 268nm (ϵ 10228). ν_{max} (KBr) 3392, 1796, 1729, 1688cm⁻¹. δ (250 MHz) 1.45 (9H,s,C(CH₃)₃), 2.03 & 2.11 (1H,dd, J=7.8 & 9.1Hz,CH₂CO₂Bu^t), 2.15 (3H,s,3-CH₃), 2.59 & 2.66 (1H,dd,J=4.3 & 17.0Hz,CH₂CO₂Bu^t), 4.00 (1H,dd,J=4.3 & 9.1Hz,2-H), 4.51 (2H,d,J=4.9Hz,6-H), 4.57 (2H,s,CH₂CON), 6.19 (1H,dd,J=4.9 & 10.4Hz, 7-H), 6.92- 7.50 (16H,m,PhO & CHPh₂), 7.94 (1H,d,J=10.4Hz,NH). (Found : C,65.20; H,5.85; N,4.12; S,5.13. C₃₅H₃₆N₂O₈S requires C,65.22; H,5.59; N,4.35; S,4.97%). m/e 667 MNa⁺.

(1S,6R,7R) 3,4-Dimethyl-7 β -phenoxyacetamidoceph-3-em 1-oxide (299).

To a stirred solution of (291) (0.8g, 1.5mmol) in dichloromethane (20ml) at 0°C was added trifluoroacetic acid (2.5g) and anisole (2.4g). Stirring was continued for 90 minutes and solvents removed *in vacuo*. The residue was dissolved in dichloromethane containing triethylamine (0.1ml) and stirred at room temperature for 12h. The solution was diluted with dichloromethane and washed with 1M HCl, saturated aqueous NaHCO₃

solution, brine, dried and solvent removed under reduced pressure to give a pale brown solid. Recrystallisation from ethyl acetate afforded (1S,6R,7R) 3,4-dimethyl-7β-phenoxyacetamidoceph-3-em 1-oxide (299) as a cream solid (0.38g, 76%). mp 199-202°C (decomp.). λ_{max} 251nm (ϵ 21166). ν_{max} (KBr) 3310, 1764, 1675 cm^{-1} . δ (d_6 -DMSO, 250 MHz) 1.71 (3H,s,3-CH₃), 2.15 (3H,s,4-CH₃), 3.47 & 3.53 (2H,ABq,J=18.2Hz,2-H), 4.67 (2H,s,CH₂CON), 4.81 (1H,d,J=4.7Hz,6-H), 5.83 (1H,dd,J=4.7 & 9.8Hz,7-H), 6.94-7.35 (5H,m,PhO), 8.16 (1H,d,J=9.8Hz,NH). (Found : C,57.43; H,5.29; N,8.38; S,9.21. C₁₆H₁₈N₂O₄S requires C,57.48; H,5.37; N,8.38; S,9.55%). m/e 335 MH⁺.

(6R,7R) 3,4-Dimethyl-7β-phenoxyacetamidoceph-3-em (300).

Sulphoxide (299) (0.27g, 0.81mmol) dissolved in DMF (20ml) at 0°C was stirred with potassium iodide (0.54g, 3.24mmol) and acetyl chloride (0.25g, 3.24mmol) for 4h. The reaction mixture was poured onto ice/water containing Na₂S₂O₅ and extracted with ethyl acetate. The organic phase was washed with saturated aqueous NaHCO₃ solution, brine, dried and solvent evaporated under reduced pressure to give a brown semi-solid which was recrystallised from ethyl acetate/petrol to afford (6R,7R) 3,4-dimethyl-7β-phenoxyacetamidoceph-3-em (300) (0.17g, 65%) as a white foam. λ_{max} 254nm (ϵ 15360). ν_{max} 3279, 1778, 1666 cm^{-1} . δ (250 MHz) 1.73 (3H,s,3-CH₃), 2.19 (3H,s,4-CH₃), 3.43 & 3.52 (2H,ABq,J=17.3Hz,2-H), 4.55 (2H,s,CH₂CON), 5.12 (1H,d,J=4.4Hz,6-H), 5.66

(1H,d,J=4.4 & 8.9Hz,7-H), 6.98-7.60 (6H,m,PhO & NH).
(Found : M⁺ 334.1014. C₁₆H₁₈N₂O₄S requires 334.0987).

Reaction of (266) with Benzyl Bromide and Sodium Hydride.

To a stirred solution of sulfoxide (266) (1.5g, 2.8mmol) in DMF (30ml) containing benzyl bromide (0.72g, 4.2mmol) at 0°C under an N₂ atmosphere was added sodium hydride (0.1g, 4.2mmol) and stirring continued for 2h. The reaction mixture was poured onto ice/water and extracted with ethyl acetate. The organic portion was washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and concentrated *in vacuo* to give a yellow oil. Chromatography on silica gel afforded firstly diphenylmethyl (1S,6R,7R) 3-methyl-4-benzyl-7β-phenoxy-acetamidoceph-2-em-4-carboxylate 1-oxide (301) as a white crystalline solid (0.69g, 40%). mp 124-126°C. λ_{max} 257nm (ε 3280). ν_{max} (KBr) 3334, 1775, 1740, 1690cm⁻¹. δ (250 MHz) 1.61 (3H,d,J=1.3Hz,3-CH₃), 3.96 & 4.12 (2H, ABq,J=14.8Hz,CH₂Ph), 4.44 (1H,d,J=4.7Hz,6-H), 4.52 & 4.59 (2H,ABq,J=15.1Hz,CH₂CON), 5.80 (1H,dd,J=4.7 & 10.6Hz,7-H), 6.54 (1H,d,J=1.3Hz,2-H), 6.93-7.42 (21H,m, PhO,CHPh₂ & CH₂Ph), 8.24 (1H,d,J=10.6Hz,NH). (Found : C,69.32; H,5.18; N,4.47; S,4.96. C₃₆H₃₂N₂O₆S requires C,69.68; H,5.16; N,4.52; S,5.16%). m/e 643 MNa⁺.

Further elution afforded diphenylmethyl (1S,6R,7R) 2,4-dibenzyl-3-methyl-7β-phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (302) as a white foam (0.35g, 20%).

λ_{max} 256nm (ϵ 4820). ν_{max} (KBr) 3355, 1759, 1739, 1694 cm^{-1} . δ (250 MHz) 1.56 (3H,s,3-CH₃), 3.71 & 3.81 (2H, ABq, J=16.1Hz, CH₂Ph), 3.97 & 4.17 (2H, ABq, J=14.2Hz, CH₂Ph) 4.41 (1H,d, J=4.4Hz, 6-H), 4.48 & 4.55 (2H, ABq, J=15.1Hz, CH₂CON), 5.74 (1H,dd, J=4.4 & 10.5Hz, 7-H), 6.92-7.46 (26H,m, PhO, CHPh₂ & 2xCH₂Ph), 8.03 (1H,d, J=10.5Hz, NH). (Found : C, 72.50; H, 5.47; N, 3.91; S, 4.42. C₄₃H₃₈N₂O₆S requires C, 72.67; H, 5.35; N, 3.94; S, 4.51%). m/e 733 MNa⁺.

(1S,6R,7R) 3-Methyl-4-benzyl-7 β -phenoxyacetamidoceph-3-em 1-oxide (303).

Sulphoxide (301) (0.3g, 0.48mmol) was stirred with trifluoroacetic acid (1.65g) and anisole (1.57g) at room temperature for 30 minutes. Solvents were removed *in vacuo* and the residue stirred in dichloromethane (15ml) containing triethylamine (0.1ml) for 18h. The solution was partitioned between dichloromethane and saturated aqueous NaHCO₃ solution, the aqueous phase was acidified with 1M HCl and extracted with dichloromethane. This organic portion was washed with brine, dried and solvent evaporated under reduced pressure to give a cream solid.

Recrystallisation from ethyl acetate/ petrol afforded

(1S,6R,7R) 3-methyl-4-benzyl-7 β -phenoxyacetamidoceph-3-em 1-oxide (303) as a flocculent white solid

(0.14g, 71%). mp 152-154°C. λ_{max} 252nm (ϵ 8200). ν_{max} (KBr) 3312, 1768, 1671 cm^{-1} . δ (d₆-DMSO, 250 MHz) 1.70 (3H,s,3-CH₃), 3.44 & 3.59 (2H, ABq, J=17.6Hz, 2-H), 3.82 &

3.99 (2H, ABq, $J=15.3\text{Hz}$, CH_2Ph), 4.56 (2H, s, CH_2CON), 4.61 (1H, d, $J=4.4\text{Hz}$, 6-H), 5.81 (1H, dd, $J=4.4$ & 9.9Hz , 7-H), 6.96-7.44 (10H, m, PhO & CH_2Ph), 8.23 (1H, d, $J=9.9\text{Hz}$, NH). (Found : C, 64.57; H, 4.98; N, 6.80; S, 7.62. $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_4\text{S}$ requires C, 64.39; H, 5.36; N, 6.83; S, 7.80%). m/e 411 MH^+ .

De-oxygenation of Sulphoxide (303).

A solution of sulphoxide (303) (0.1g, 0.24mmol) in DMF (20ml) was stirred at ice temperature with acetyl chloride (0.07g, 0.96mmol) and potassium iodide (0.16g, 0.96mmol) for 3h. The reaction mixture was poured onto ice/water containing $\text{Na}_2\text{S}_2\text{O}_5$ and extracted with ethyl acetate. The organic portion was washed with saturated aqueous NaHCO_3 solution, brine, dried and solvent removed *in vacuo*. Chromatography afforded as the only β -lactam product (6R,7R) 3-methyl-4-benzyl-7 β -phenoxy-acetamidoceph-3-em (304) as a colourless oil (33mg, 35%). λ_{max} 254nm (ϵ 7324). ν_{max} (film) 3278, 1760, 1675cm^{-1} . δ (400 MHz) 1.72 (3H, s, 3- CH_3), 3.42 & 3.58 (2H, ABq, $J=18.0\text{Hz}$, 2-H), 3.90 & 4.08 (2H, ABq, $J=15.2\text{Hz}$, CH_2Ph), 4.58 (2H, s, CH_2CON), 4.86 (1H, d, $J=4.6\text{Hz}$, 6-H), 5.70 (1H, dd, $J=4.6$ & 8.6Hz , 7-H), 6.86-7.25 (10H, m, PhO & CH_2Ph), 8.00 (1H, d, $J=8.6\text{Hz}$, NH). m/e 417 MNa^+ .

Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7 β -phenoxy-acetamidoceph-3-em-4-carboxylate with Sodium Hydride and Iodomethane.

Cephem (153) (2.06g, 4mmol) in DMF (80ml) was stirred at 0°C with iodomethane (0.85g, 6mmol) for 10 minutes, followed by addition of sodium hydride (0.14g, 6mmol) and stirring was continued for 1h. The reaction mixture was poured onto ice/water and extracted with ethyl acetate. The organic portion was washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and concentrated *in vacuo* to a yellow oil. Column chromatography afforded diphenylmethyl 3,4-dimethyl-7 β -phenoxyacetamidoceph-2-em-4-carboxylate (305) as a colourless oil (1.32g, 62%). λ_{max} 258nm (ϵ 4800). ν_{max} 3325, 1772, 1748, 1692cm⁻¹. δ (250 MHz) 1.71 (3H,d,J=1.2Hz,3-CH₃), 1.98 (3H,s,4-CH₃), 4.59 (2H,s,CH₂CON), 5.16 (1H,d,J=4.5Hz,6-H), 6.22 (1H,dd,J=4.5 & 9.2Hz,7-H), 6.46 (1H,d,J=1.2Hz,2-H), 6.89-7.41 (17H,m,PhO,CHPh₂ & NH). (Found : C,67.86; H,5.38; N,5.45; S,6.56. C₃₀H₂₈N₂O₅S requires C,68.18; H,5.30; N,5.30; S,6.06%). m/e 551 MNa⁺.

Oxidation of (305).

A solution of sulphide (305) (1.32g, 2.5mmol) in dichloromethane (60ml) was stirred with *m*-CPBA (0.60g, 80% pure hence 2.6mmol) at 0°C for 1h. The reaction mixture was filtered and the filtrate partitioned

between 10% w/v aqueous $\text{Na}_2\text{S}_2\text{O}_5$ solution and dichloromethane. The organic phase was washed with saturated aqueous NaHCO_3 solution, brine, dried and solvent removed under reduced pressure to yield a yellow oil. Crystallisation from ethyl acetate/petrol afforded a white crystalline solid (1.27g, 94%) which was found to be identical in all respects to a previously prepared sample of sulfoxide (291).

Attempted Reaction of α -Sulfoxide (306) with Sodium Hydride and Iodomethane.

α -Sulfoxide (306) (0.53g, 1mmol) was stirred in DMF (30ml) containing iodomethane (0.17g, 1.2mmol) with sodium hydride (0.03g, 1.2mmol) at 0°C for 4h. The solution was poured onto ice/water, extracted with ethyl acetate and the organic portion washed with 1M HCl, saturated aqueous NaHCO_3 solution, brine, dried and concentrated *in vacuo*. TLC indicated a complex mixture containing polar components which was not further investigated.

Attempted Reaction of (153) with Ethyl Bromoacetate.

The ester (153) (0.51g, 1mmol) in DMF (20ml) containing ethyl bromoacetate (0.33g, 2mmol) was stirred at 0°C with sodium hydride (0.07g, 1.25mmol) for 10h. No trace of reaction was observed by tlc.

Attempted Reaction of (153) with Methyl 3-Bromo-propionate.

Under similar conditions to those employed above, (153) (0.51g, 1mmol) was reacted with sodium hydride (0.07g, 1.25mmol) in the presence of methyl 3-bromopropionate (0.33g, 2mmol). A smell of methyl acrylate was noted, however no trace of reaction was observed after 6h at 0°C and (153) was recovered in quantitative yield.

Reaction of Diphenylmethyl (6R,7R) 3-Methyl-7 β -phenoxy-acetamidoceph-3-em-4-carboxylate (153) with LDA and Iodomethane.

To a stirred solution of diisopropylamine (0.202g, 2mmol) in THF (20ml) at 0°C under an N₂ atmosphere was added *n*-butyl lithium (1.28ml, 1.6M solution in hexane) and stirring continued for 10 minutes before cooling to -70°C. Cephem (153) (0.51g, 1mmol) in THF (10ml) was added dropwise to the basic solution over 5 minutes, then the solution stirred for 15 minutes. Iodomethane (0.28g, 2mmol) was added in one portion, followed by stirring for a further 90 minutes. The reaction mixture was allowed to warm to room temperature then poured onto ice-cold saturated aqueous NH₄Cl solution and extracted with ethyl acetate. The organic portion was washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo*. Chromatography gave a

single β -lactam containing product as a pale yellow foam. PMR spectroscopy indicated the presence of two compounds which could not be separated by chromatography.

In order to overcome the separation difficulties, the crude product was dissolved in dichloromethane (40ml) and treated with *m*-CPBA until no unoxidised material remained. After work-up, chromatography afforded firstly diphenylmethyl (1S,6R,7R) 3,4-dimethyl-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (291) as a white powder (0.21g, 48%). MP, tlc, ir and pmr showed this to be identical to a previously prepared sample.

Further elution resulted in diphenylmethyl (1R,6R,7S) 3,4-dimethyl-7 α -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (328) as an amorphous white solid (0.09g, 16%). mp 184-186°C. λ_{max} 252nm (ϵ 8400). ν_{max} 3267, 1777, 1738, 1707cm⁻¹. δ (250 MHz) 1.76 (3H,s,3-CH₃), 2.07 (3H,s,4-CH₃), 4.50 (2H,s,CH₂CON), 4.80 (1H,d,J=1.7Hz, 6-H), 5.18 (1H,dd,J=1.7 & 6.5Hz,7-H), 6.64 (1H,s,2-H), 6.89-7.37 (17H,m,PhO,CHPh₂ & NH). (Found : C,66.40; H,5.23; N,4.91; S,5.82. C₃₀H₂₈N₂O₆S requires C,66.17; H,5.15; N,5.15; S,5.88%). m/e 567 MNa⁺.

Reaction of (153) with LDA.

A solution of diisopropylamine (0.202g, 2mmol) in THF (20ml) at 0°C was stirred under an N₂ atmosphere with *n*-butyl lithium (1.28ml, 1.56M solution in hexane) for 5

minutes before cooling to -70°C . To this basic solution was added cephem (**153**) (0.51g, 1mmol) in THF (10ml) over 5 minutes followed by stirring at -70°C for 100 minutes. The solution was allowed to warm to room temperature then poured onto ice-cold saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic portion was washed with 1M HCl, saturated aqueous NaHCO_3 solution, brine, dried and solvent removed *in vacuo* to yield a light brown foam (0.6g). TLC indicated the formation of an equilibrium mixture of Δ -2 and Δ -3 isomers (**335**) and (**153**) (ca. 1:2).

Oxidation of the crude foam with *m*-CPBA gave, after work-up, a white crystalline solid (0.45g, 85%) which was identical in all respects to an authentic sample of sulphoxide (**266**).

Reaction of Ceph-2-em (**305**) with LDA.

To a stirred solution of diisopropylamine (0.202g, 2mmol) in THF (15ml) at 0°C under an N_2 atmosphere was added *n*-butyl lithium (1.28ml, 1.56M solution in hexane). Stirring at 0°C was continued for 5 minutes before cooling to -70°C . A solution of ceph-2-em (**305**) (0.264g, 0.5mmol) in THF (8ml) was added over 5 minutes, and stirred at -70°C for 100 minutes. The reaction mixture was poured onto ice-cold saturated aqueous NH_4Cl solution, extracted with ethyl acetate and the organic layer washed with 1M HCl, saturated aqueous NaHCO_3 solution, brine, dried and solvents removed under

reduced pressure to afford a colourless oil (0.25g).

The oil was taken up in dichloromethane and oxidised with *m*-CPBA until no unoxidised material remained. The reaction mixture was washed with 10% w/v aqueous Na₂S₂O₅ solution, saturated aqueous NaHCO₃ solution, brine, dried and concentrated *in vacuo* to give a white foam (0.16g). Preparative tlc yielded unreacted starting material (**291**) (0.10g, 37%) as the least polar material.

The second component, obtained as a white powder (0.06g, 22%), was found to be identical in all respects to a sample of 7-epicephem (**328**) obtained above.

Diphenylmethyl (1R,6R,7R) 3,4-Dimethyl-7 β -phenoxy-acetamidoceph-2-em-4-carboxylate 1-oxide (**336**).

To a stirred solution of diisopropylamine (0.28ml, 2mmol) in dry THF (20ml) at 0°C under a nitrogen atmosphere was added *n*-butyl lithium (1.28ml, 1.56M solution in hexane) and stirring continued for 5 minutes before cooling to -70°C. A solution of α -sulphoxide (**306**) in THF (10ml) and HMPA (2ml) was added dropwise to the basic solution over 10 minutes, and after a further 25 minutes iodomethane (0.28g, 2mmol) was added in one portion. Stirring at -70°C was continued for 3h then the solution allowed to warm to room temperature. The reaction mixture was poured onto ice-cold saturated NH₄Cl solution and extracted with ethyl acetate. The organic portion was washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent

evaporated under reduced pressure. Silica gel chromatography of the resulting foam afforded diphenylmethyl (1R,6R,7R) 3,4-dimethyl-7 β -phenoxy-acetamidoceph-2-em-4-carboxylate 1-oxide (336) as white crystals (0.27g, 50%). mp 202-205°C. λ_{max} 254nm (ϵ 2817). ν_{max} (KBr) 3286, 1783, 1737, 1678 cm^{-1} . δ (250 MHz) 1.74 (3H,d,J=1.1Hz,3-CH₃), 1.91 (3H,s,4-CH₃), 4.57 (2H,s,CH₂CON), 4.70 (1H,d,J=4.1Hz,6-H), 5.41 (1H, dd,J=4.1 & 8.0Hz,7-H), 6.18 (1H,d,J=1.1Hz,2-H), 6.92-7.42 (17H,m,PhO,CHPh₂ & NH). (Found : C,66.09; H,5.04; N,5.11; S,5.65. C₃₀H₂₈N₂O₆S requires C,66.17; H,5.15; N,5.15; S,5.88%). m/e 567 MNa⁺.

Reaction of Sulphoxide (266) with LDA and Iodomethane.

A solution of diisopropylamine (0.202g, 2mmol) in dry THF (20ml) was stirred at 0°C under an N₂ atmosphere with *n*-butyl lithium (1.28ml, 1.56M solution in hexane) for 10 minutes, then cooled to -70°C. To the stirred solution was added dropwise sulphoxide (266) in dry THF (10ml) and HMPA (2ml) over 5 minutes and stirred at -70°C for 20 minutes. Iodomethane (0.28g, 2mmol) was added and the solution stirred at -70°C for 2h, then allowed to warm to room temperature. The reaction mixture was poured onto ice-cold saturated NH₄Cl solution and extracted with ethyl acetate. The organic portion was washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo* to give a light brown oil. Column chromatography afforded

firstly diphenylmethyl (1S,6R,7R) 2,2,3-trimethyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (293) (0.26g, 47%) which was identical in all respects to a sample of (293) prepared above.

Further elution resulted in diphenylmethyl (1S,6R,7R) 3,4-dimethyl-7 β -phenoxyacetamidoceph-2-em-4-carboxylate 1-oxide (291) (0.075g, 14%) identical to an authentic sample.

Attempted Reaction of (153) with LDA and Acetyl Chloride.

A solution of diisopropylamine (0.202g, 2mmol) in THF (20ml) was stirred at 0°C under an N₂ atmosphere for 5 minutes with *n*-butyl lithium (1.28ml, 1.56M solution in hexane), followed by cooling to -70°C. Addition of cephem (153) (0.51g, 1mmol) in THF over 5 minutes was followed by stirring for 30 minutes, then acetyl chloride (0.16g, 2mmol) was added in one portion. The reaction was monitored by tlc, and after 5h at -70°C no reaction was observed. Quenching of the reaction followed by work-up as detailed above resulted in recovery of unreacted (153) in 90% yield.

Diphenylmethyl (6R,7R) 3-Methyl-4-(*t*-butyloxycarbonyl)methyl-7 β -phenoxyacetamidoceph-2-em-4-carboxylate (345).

Diisopropylamine (0.202g, 2mmol) in THF (20ml) was stirred at 0°C under an N₂ stream with *n*-butyl lithium (1.1ml, 1.78M solution in hexane) for 5 minutes before

cooling to -70°C . Cephem (**153**) (0.51g, 1mmol) in THF (12ml) was added dropwise and the resulting red solution stirred at -70° for 30 minutes before *t*-butyl bromoacetate (0.39g, 2mmol) was added and stirring continued for 3h. The reaction mixture was allowed to warm to 0°C , poured onto ice-cold saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic portion was washed with saturated aqueous NaHCO_3 solution, brine, dried and solvent evaporated *in vacuo* to give a brown oil. Chromatography yielded diphenylmethyl (6R,7R) 3-methyl-4-(*t*-butyloxycarbonyl) methyl-7 β -phenoxyacetamidoceph-2-em-4-carboxylate (**345**) as a pale yellow foam (0.28g, 44%). λ_{max} 255nm (ϵ 3500). ν_{max} (KBr) 3416, 1775, 1734, 1696 cm^{-1} . δ (250 MHz) 1.39 (9H,s, $\text{C}(\text{CH}_3)_3$), 1.74 (3H,d, $J=1.2\text{Hz}$,3- CH_3), 3.13 & 3.57 (2H,ABq, $J=17.1\text{Hz}$, $\underline{\text{CH}_2}\text{CO}_2\text{Bu}^t$), 4.54 (2H,s, CH_2CON), 5.53–5.62 (2H,m,6-H & 7-H), 6.02 (1H,d, $J=1.2\text{Hz}$,2-H), 6.89–7.40 (17H,m,PhO, CHPh_2 & NH). (Found : C,66.62; H,5.72; N,4.08; S,5.33. $\text{C}_{35}\text{H}_{36}\text{N}_2\text{O}_7\text{S}$ requires C,66.88; H,5.73; N,4.46; S,5.09%). m/e 651 MNa^+ .

Attempted Reaction of (**345**) with LDA and Iodomethane.

To a solution of diisopropylamine (0.072g, 0.72mmol) in THF (10ml) at 0°C under an N_2 atmosphere was added *n*-butyl lithium (0.44ml, 1.56M solution in hexane) and stirring continued for 5 minutes. The basic solution was cooled to -70°C and cep-2-em (**345**) (0.23g, 0.36mmol) in THF (5ml) was added dropwise. Stirring at

-70° was maintained for 4h, then the solution quenched by pouring onto saturated aqueous NH₄Cl solution and extracted with ethyl acetate. The organic portion was washed with 1M HCl, saturated aqueous NaHCO₃ solution, brine, dried and solvent removed *in vacuo* to afford unreacted (345) in quantitative yield.

Attempted Reaction of (153) with Methyl Glycidate.

To a stirred solution of diisopropylamine (0.202g, 2mmol) in THF (20ml) at 0°C under an N₂ atmosphere was added *n*-butyl lithium (0.28ml, 1.56M solution in hexane) and stirring continued for 5 minutes at 0°C before cooling to -70°C. Cephem (153) (0.51g, 1mmol) in THF (8ml) was added dropwise to the basic solution and stirred for 20 minutes before methyl glycidate (0.20g, 2mmol) in THF (4ml) was added. Stirring was continued at -70°C for 3h, then the solution warmed to 0°C. Work-up as before afforded an equilibrium mixture of Δ-2 and Δ-3 cepheems (ca. 1:3) as observed on tlc.

Attempted Reaction of (53) with Benzaldehyde in Toluene.

A solution of sulphoxide (55) (0.5g, 1mmol) in toluene (30ml) containing triethylamine (0.1ml) and benzaldehyde (0.1g, 1mmol) was heated at reflux for 15h. Solvent was evaporated *in vacuo* to give a black oil which was taken up in ethyl acetate and washed with 1M HCl, brine, dried and concentrated under reduced pressure to give a dark brown oil. TLC indicated only baseline material and ir

showed the absence of the β -lactam carbonyl stretch.

The reaction was repeated on the same scale as above under an N_2 atmosphere with a Dean & Stark separator. After 8h complete conversion of starting material to baseline components was observed by tlc, and ir indicated loss of the β -lactam carbonyl stretching frequency.

Attempted Reaction of (55) with Benzaldehyde in Acetonitrile.

A solution of ester (55) (0.5g, 1mmol) in acetonitrile (25ml) containing triethylamine (0.1ml) and benzaldehyde (0.1g, 1mmol) was stirred at room temperature for 12h. Work-up as described above gave a black oil (0.58g) which was found by ir to have no β -lactam carbonyl stretch.

Repetition of the reaction at 0°C and at reflux on the same scale resulted in similar decomposition of the starting material.

When (55) was refluxed in acetonitrile containing benzaldehyde (0.1g) and triethylamine (0.1ml) in the presence of molecular sieve 4A, no reaction was observed by tlc. Continued reflux for 8h did not result in decomposition and unreacted starting material was still present.

Trichloroethyl (1S,6R,7R) 2 α -Bromo-3-methyl-7 β -phenoxy-acetamidoceph-3-em-4-carboxylate 1-oxide (362).

To a stirred solution of sulphoxide (55) (0.2g, 0.4mmol) in dichloromethane (20ml) at -15°C under a nitrogen atmosphere was added triethylamine (0.061g, 0.6mmol) and N-bromosuccinimide (0.077g, 0.6mmol). After 30 minutes, tlc indicated no starting material remained and the red solution was washed with 1M HCl, brine, dried and solvent removed *in vacuo*.

Chromatography afforded trichloroethyl (1S,6R,7R) 2 α -bromo-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (362) (0.21g, 91%) as a cream foam. λ_{max} 265nm (ϵ 12420). ν_{max} 3382, 1802, 1743, 1695cm⁻¹. δ (400 MHz) 2.33 (3H,s,3-CH₃), 4.59 (2H,s,CH₂CON), 4.92 & 4.99 (2H,ABq,J=12.0Hz,CH₂CCl₃), 5.15 (1H,s,2-H), 5.24 (1H,d,J=4.8Hz,6-H), 6.24 (1H,dd,J=4.8 & 10.2Hz,7-H), 6.92-7.34 (5H,m,PhO), 7.77 (1H,d,J=10.2Hz,NH). (Found : C,37.22; H,2.51; N,4.50; S,5.92. C₁₈H₁₆N₂BrCl₃O₆S requires C,37.56; H,2.78; N,4.87; S,5.56%). m/e 573 and 575 both MH⁺.

Attempted Reactions of the Bromo Sulphoxide (362).

a) De-oxygenation - Sulphoxide (362) (0.20g, 0.35mmol) dissolved in DMF (10ml) was stirred at 0°C with PBr₃ (0.1ml, 1.05mmol) for 5h. The solution was poured onto ice/water, extracted with ethyl acetate and the organic portion washed with saturated aqueous NaHCO₃ solution,

brine, dried and solvent removed under reduced pressure to give a light brown foam (0.15g) which was identical (tlc,pmr,uv) to unreacted (362).

Acetyl chloride/potassium iodide also failed to result in de-oxygenation of the sulphoxide function.

b) Methyl Vinyl Ketone - A solution of cephem (362) (0.34g, 0.6mmol) in methyl vinyl ketone (10ml) was stirred with triethylamine (0.05ml) at room temperature for 18h. TLC showed degradation of (362) to baseline material and after work-up, ir denoted loss of the β -lactam carbonyl stretching frequency.

The use of Triton B, sodium hydride and LDA as base all resulted in similar decomposition.

c) Triphenylphosphine - A solution of sulphoxide (362) (0.2g, 0.35mmol) in dichloroethane (20ml) was heated at reflux with triphenylphosphine (0.10g, 0.4mmol) for 6h. The mixture was diluted with further dichloroethane and washed with brine, dried and solvent removed *in vacuo*. IR indicated loss of the β -lactam carbonyl function.

Trichloroethyl (1S,6R,7R) 2-bis(Thiomethyl)methylene -3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (381).

To a stirred solution of sulphoxide (55) (0.5g, 1mmol) in DMSO (20ml) containing carbon disulphide (0.25ml, 4.2mmol) was added sodium hydride (0.06g, 2.5mmol) in

one portion. Stirring at room temperature was continued for 30 minutes, then iodomethane (0.36g, 2.5mmol) was added followed by further stirring for 2h. The reaction mixture was poured onto ice/water and extracted with ethyl acetate. The organic portion was washed with 1M HCl, brine, dried and solvent removed *in vacuo*.

Chromatography afforded trichloroethyl (1S,6R,7R) 2-bis(thiomethyl)methylene-3-methyl-7 β -phenoxy-acetamidoceph-3-em-4-carboxylate 1-oxide (381) as a yellow solid (0.42g, 61%). mp 158-161°C. λ_{max} 281nm (ϵ 9820). ν_{max} 3372, 1796, 1737, 1695cm⁻¹. δ (400 MHz) 2.46 (6H,s,2 x SCH₃), 2.52 (3H,s,3-CH₃), 4.48 (1H,d,J=4.8Hz, 6-H), 4.59 (2H,s,CH₂CON), 4.87 & 5.06 (2H,ABq,J=12.0Hz, CH₂CCl₃), 6.09 (1H,dd,J=4.8 & 10.3Hz,7-H), 6.95-7.33 (5H,m,PhO), 7.86 (1H,d,J=10.3Hz,NH). (Found : C,42.34; H,3.59; N,4.31; S,15.88. C₂₁H₂₁N₂Cl₃O₆S₃ requires C,42.04; H,3.50; N,4.67; S,16.01%). m/e 621 MNa⁺.

Diphenylmethyl (1S,6R,7R) 2-bis(Thiomethyl)methylene-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (382).

A solution of cephem (266) (0.53g, 1mmol) in DMSO (20ml) containing carbon disulphide (0.25ml, 4.2mmol) was stirred with sodium hydride (0.06g, 2.5mmol) for 30 minutes. Iodomethane (0.36g, 2.5mmol) was added and stirring continued for a further 1h, then the reaction mixture poured onto ice/water and extracted with ethyl acetate. The organic portion was washed with 1M HCl,

brine, dried and solvent evaporated under reduced pressure to give an orange semi-solid.

Recrystallisation from ethyl acetate afforded diphenylmethyl (1S,6R,7R) 2-bis(thiomethyl)methylene-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (382) as a bright yellow solid (0.39g, 60%). mp 197-198°C. λ_{max} 369nm (ϵ 6830). ν_{max} 3307, 1785, 1728, 1687cm⁻¹. δ (250 MHz) 2.33 (3H,s,SCH₃), 2.40 (3H,s,CH₃), 2.50 (3H,s,3-CH₃), 4.44 (1H,d,J=4.8Hz,6-H), 4.59 (2H,s,CH₂CON), 6.06 (1H,dd,J=4.8 & 10.4Hz,7-H), 6.95-7.50 (16H,m,PhO & CHPh₂), 7.91 (1H,d,J=10.4Hz,NH). (Found : C,60.73; H,4.65; N,4.41; S,15.00. C₃₂H₃₀N₂O₆S₃ requires C,60.57; H,4.73; N,4.42; S,15.14%). m/e 657 MNa⁺.

Trichloroethyl (1S,6R,7R) 2-[2'-(1,3-Dithiolylidene)]-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (383).

A solution of cephem (55) (0.5g, 1mmol) in DMSO (25ml) containing carbon disulphide (0.32g, 4.2mmol) was stirred under an N₂ atmosphere with sodium hydride (0.06g, 2.5mmol) for 45 minutes. 1,2-Dibromoethane (0.47g, 2.5mmol) was added in one portion and the resulting red solution stirred for 4h. The solution was poured onto ice-cold brine, extracted with ethyl acetate and the organic portion washed with 1M HCl, brine, dried and concentrated *in vacuo*. Chromatography afforded trichloroethyl (1S,6R,7R) 2-[2'-(1,3-dithiolylidene)]-

3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (383) as an orange powder (0.20g, 42%). mp 165-167°C. λ_{max} 359nm (ϵ 7345). ν_{max} (KBr) 3416, 1777, 1740, 1696cm⁻¹. δ (250 MHz) 2.46 (3H,s,3-CH₃), 3.56-3.83 (4H,m,SCH₂CH₂S), 4.71 (2H,s,CH₂CON), 4.98 & 5.19 (2H,ABq,J=12.2Hz,CH₂CCl₃), 5.06 (1H,d,J=4.7Hz,6-H), 6.00 (1H,dd,J=4.7 & 9.6Hz,7-H), 6.94-7.36 (5H,m,PhO), 8.18 (1H,d,J=9.6Hz,NH). (Found : C,42.58; H,3.37; N,4.58; S,15.90. C₂₁H₁₉N₂Cl₃O₆S₃ requires C,42.18; H,3.18; N,4.68; S,16.06%). m/e 619 MNa⁺.

Trichloroethyl (1S,6R,7R) 2-(Anilino-thiomethyl)methylene-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (384).

A solution of cephem (55) (0.5g, 1mmol) in DMSO (25ml) containing phenyl isothiocyanate (0.27g, 2mmol) at room temperature was stirred under an N₂ atmosphere with sodium hydride (0.04g, 1.5mmol) for 40 minutes.

Iodomethane (0.21g, 1.5mmol) was added and stirring continued for a further 2h. The yellow solution was poured onto ice-cold brine, extracted with ethyl acetate and the organic portion washed with 1M HCl, brine, dried and solvent evaporated *in vacuo* to give a yellow solid.

Chromatography afforded trichloroethyl (1S,6R,7R) 2-(anilino-thiomethyl)methylene-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylate 1-oxide (384)

as a bright yellow solid (0.51g, 79%). mp 138-141°C.

λ_{max} 285nm (ϵ 10370), 387nm (ϵ 20440). ν_{max} 3376, 1791,

1757, 1693 cm^{-1} . δ (250 MHz) 2.25 (3H,s,SCH₃), 2.45 (3H,s,3-CH₃), 4.52 (1H,d,J=4.5Hz,6-H), 4.57 (2H,s,CH₂CON), 4.78 & 5.02 (2H,ABq,J=12.0Hz,CH₂CCl₃), 6.21 (1H,dd,J=4.5 & 10.5Hz,7-H), 6.74-7.34 (11H,m,PhO & NHPH), 8.04 (1H,d,J=10.5Hz,CONH). (Found : C,48.20; H,3.82; N,6.23; S,9.55. C₂₆H₂₄N₃Cl₃O₆S₂ requires C,48.41; H,3.72; N,6.52; S,9.93%). m/e 668 MNa⁺.

Reaction of (55) with Sodium Hydride, Iodomethane and Carbon Disulphide in THF.

To a stirred solution of sulphoxide (55) (0.5g, 1mmol) in THF (20ml) containing carbon disulphide (0.25ml, 4.2mmol) was added sodium hydride (0.06g, 2.5mmol) in one portion. Stirring at room temperature was continued for 30 minutes, then iodomethane (0.36g, 2.5mmol) was added followed by further stirring for 2h. The reaction mixture was poured onto ice/water and extracted with ethyl acetate. The organic portion was washed with 1M HCl, brine, dried and solvent removed *in vacuo*. Chromatography afforded a single product (0.15g, 25%) which was identical in all respects to a previously prepared sample of (381).

De-oxygenation of (381).

Sulphoxide (381) (0.21g, 0.35mmol) in DMF (15ml) was stirred at 0°C with potassium iodide (0.23g, 1.4mmol) and acetyl chloride (0.11g, 1.4mmol) for 3h. The solution was poured onto ice/water containing Na₂S₂O₅,

extracted with ethyl acetate and the organic phase washed with saturated aqueous NaHCO_3 solution, brine, dried and solvent removed *in vacuo* to give a brown oil. Chromatography afforded trichloroethyl (6R,7R) 2-bis(thiomethyl)methylene-3-methyl-7 β -phenoxy acetamidoceph-3-em-4-carboxylate (387) as a yellow oil (0.10g, 81%). λ_{max} 295nm (ϵ 7540). ν_{max} (film) 3316, 1783, 1728, 1688 cm^{-1} . δ (250 MHz) 2.28 (3H,s, SCH_3), 2.34 (3H,s, SCH_3), 2.67 (3H,s,3- CH_3), 4.58 & 4.65 (2H,ABq,J=15.0Hz, CH_2CON), 4.83 & 4.89 (2H,ABq,J=12.1Hz, CH_2CCl_3), 5.43 (1H,d,J=4.3Hz,6-H), 5.77 (1H,dd,J=4.3 & 9.0Hz,7-H), 6.97-7.39 (5H,m,PhO), 7.59 (1H,d,J=9.0Hz,NH). (Found : C,43.29; H,3.42; N,4.63; S,16.50. $\text{C}_{21}\text{H}_{21}\text{N}_2\text{Cl}_3\text{O}_5\text{S}_3$ requires C,43.18; H,3.60; N,4.80; S,16.45%). m/e 585 MH^+ .

Reaction of (387) with Zinc and Acetic Acid.

To a solution of sulphide (387) (0.20g, 0.34mmol) in DMF and glacial acetic acid (10ml, 25:7.5 v/v) was added powdered zinc (0.6g) and the suspension stirred at room temperature for 1h. Zinc was removed by filtration and the filtrate partitioned between ethyl acetate and saturated aqueous NaHCO_3 solution. The aqueous portion was acidified with 1M HCl and extracted with ethyl acetate. This organic phase was washed with brine, dried and concentrated *in vacuo*. TLC showed several very polar components which could not be isolated by preparative tlc.

Reaction of (382) with Zinc and Acetic Acid.

A solution of ester (382) (0.20g, 0.31mmol) in DMF and glacial acetic acid (10ml, 25:7.5 v/v) was stirred at room temperature with zinc powder (0.5g) for 2h. The reaction mixture was filtered and the filtrate diluted with ethyl acetate, washed with saturated aqueous NaHCO₃ solution, brine, dried and solvent evaporated under reduced pressure to give a yellow oil. Preparative tlc afforded diphenylmethyl (1S,6R,7R) 3-methyl-7 β -phenoxy-acetamidoceph-3-em-4-carboxylate 1-oxide (266) as the only β -lactam containing product (30mg, 18%).

(1S,6R,7R) 2-Bis(thiomethyl)methylene-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylic acid (389).

Ester (382) (0.40g, 0.6mmol) was stirred with anisole (2.0g) and trifluoroacetic acid (2.0g) at room temperature for 15 minutes. Solvents were removed *in vacuo*, the residue taken up in ethyl acetate and washed with saturated aqueous NaHCO₃ solution. The aqueous portion was acidified with HCl, extracted with ethyl acetate and this organic phase washed with brine, dried and solvent removed under reduced pressure to give a pale yellow solid which was recrystallised from ethyl acetate to yield (1S,6R,7R) 2-bis(thiomethyl)methylene-3-methyl-7 β -phenoxyacetamidoceph-3-em-4-carboxylic acid (389) (0.09g, 32%). mp 218-219°C. λ_{max} 311nm (ϵ 4680). ν_{max} (KBr) 3316, 1792, 1717,

1685 cm^{-1} . δ (d_6 -DMSO, 250 MHz) 2.24 (3H,s,SCH₃), 2.41 (3H,s,SCH₃), 2.53 (3H,s,3-CH₃), 4.71 (2H,s,CH₂CON), 5.01 (1H,d,J=4.9Hz,6-H), 5.95 (1H,dd,J=4.9 & 9.7Hz,7-H), 6.95-7.36 (5H,m,PhO), 8.17 (1H,d,J=9.7Hz,NH). m/e 491 MNa⁺.

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APPENDIX

NEW METHODOLOGY FOR C-4 SUBSTITUTION AND SULPHOXIDE DE-OXYGENATION REACTIONS IN CEPH-3-EMS.

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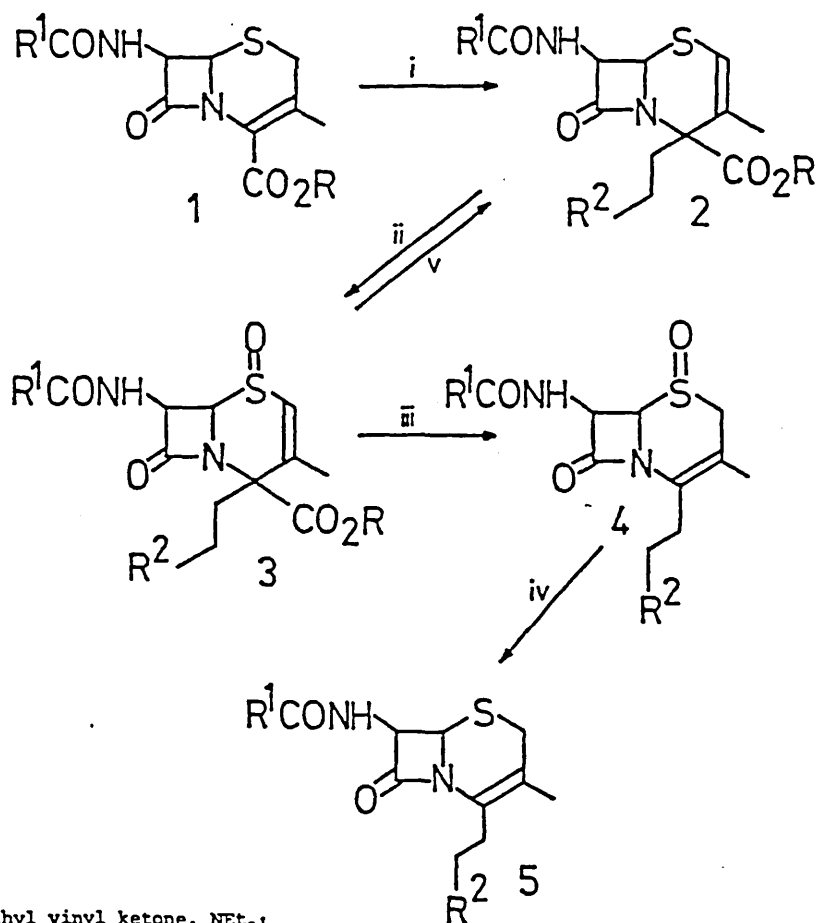
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SUMMARY: Addition of Michael acceptors to the C-4 position in ceph-3-ems followed by oxidation, de-esterification, and de-carboxylation results in the formation of new 4-monosubstituted ceph-3-ems; an unusually mild de-oxygenation process for ceph-2-em sulphoxides is also described.

The introduction of new functional groups at the C-4 position of ceph-3-ems has been achieved, almost exclusively, by manipulation of the C-4 carboxylic acid group. Thus the C-4 acid functionality has been converted into the acid chloride and thence to a number of polar groupings via the diazoketone^{1a,b}. Furthermore, cephalosporin-4-aldehyde derivatives formed by Moffatt oxidation of the corresponding 4-hydroxymethylcephalosporins have been transformed into acrylic acid derivatives which are 4-vinylogues of the parent cephalosporins². However, as C-4 disubstituted ceph-3-ems are easily prepared³, we believed that de-esterification, followed by de-carboxylation would lead to novel C-4 monosubstituted products. We now describe new synthetic methodology which provides ceph-2-ems with substituted ethyl groups attached directly to C-4 in place of the original carboxyl group. In addition we report a surprisingly facile procedure for the de-oxygenation of ceph-2-em sulphoxides.

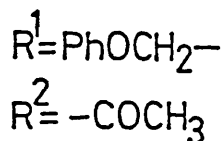
When (6R,7R)-diphenylmethyl-3-methyl-7-phenoxyacetamidoceph-3-em-4-carboxylate (1,R-CHPh₂) was treated with a catalytic amount of triethylamine in methyl vinyl ketone as reactant and solvent, it was smoothly transformed into a slightly more polar product. After rapid short-path chromatography the Michael adduct (2,R-CHPh₂), which crystallised from petrol/ethyl acetate, was obtained in 90% yield⁴. Removal of the diphenylmethyl ester function with anisole-trifluoroacetic acid⁵ afforded an 80% yield of the expected carboxylic acid (2,R-H)⁶ which did not de-carboxylate when treated with base at room temperature.

Oxidation of sulphide (2,R-CHPh₂) with *m*-chloroperoxybenzoic acid gave the sulphoxide (3,R-CHPh₂; 85% yield) which was smoothly de-esterified (anisole-trifluoroacetic acid) to produce the corresponding carboxylic acid as an unstable crystalline solid (3,R-H; 77%). When (3,R-H) was stirred in acetone containing a catalytic amount of triethylamine a flocculent white precipitate appeared almost immediately. After continued stirring overnight, the de-carboxylated ceph-3-em (4; 87% yield) was isolated⁸. The driving force for this reaction is the greater thermodynamic stability of β,γ -unsaturated sulphoxides over the corresponding α,β -unsaturated sulphoxides⁹, with the possible involvement of the sulphoxide oxygen in the de-carboxylation sequence.



Reagents:

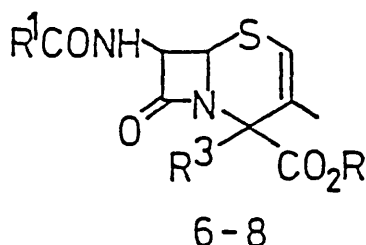
- (i) Methyl vinyl ketone, NEt_3 ;
- (ii) m -CPBA, MDC;
- (iii) Anisole-TFA;
- (iv) $\text{P}_2\text{S}_5/\text{Py}$;
- (v) Zn-HOAc .



De-oxygenation of ketone (4) with PBr_3/DMF gave a complex reaction mixture but $\text{P}_2\text{S}_5/\text{pyridine}$ in dichloromethane¹⁰ completed the sequence to afford the sulphide (5; 70%)¹¹. Other Michael acceptors which have been added at C-4 and hence converted into new C-4 monosubstituted products include acrylonitrile, dimethyl butynedioate, and methyl acrylate.

While investigating the chemistry of C-4 disubstituted compounds, we discovered an unusually mild de-oxygenation procedure for ceph-2-em sulphoxides. When a solution of (3, $\text{R}=\text{CHPh}_2$) was stirred for several hours with zinc in DMF containing glacial acetic acid¹² it was converted to the sulphide (2, $\text{R}=\text{CHPh}_2$; 89%). No ceph-3-em sulphoxides were found to de-oxygenate under similar conditions. Normal de-oxygenation of ceph-3-em sulphoxides requires activating agents such as acetyl chloride used in conjunction with

reducing agents. This has been attributed to the enhanced stability of ceph-3-em sulphoxides due to electronic factors¹³. Obviously, no such electronic effects exist in ceph-2-em sulphoxides, as the sulphides (6,R-CHPh₂;R³-CH₂CH₂CN;95% foam), and (7,R-CHPh₂;R³-CH₂CH₂CO₂CH₃;80% oil) were easily prepared by zinc-acetic acid treatment of the corresponding sulphoxides. When the oxidised adduct from cephem (1,R-CHPh₂) and dimethyl butynedioate was treated under these reducing conditions, simultaneous sulphoxide de-oxygenation and di-ester double bond reduction occurred to produce (8,R-CHPh₂;R³-CH(CO₂CH₃)CH₂CO₂CH₃;75%)¹⁴.



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4. Compound 2(R-CHPh₂); m.p. 108-109°C; $\nu_{\text{max}}(\text{KBr})$ 1770, 1742, 1710 and 1690 cm^{-1} ; $\delta(\text{CDCl}_3)$ 1.75(3H,d,J-1.0 Hz), 2.09(3H,s), 2.32-2.93(4H,m), 4.56(2H,s), 5.22(1H,d,J-4.5 Hz), 5.49(1H,dd,J-4.5 and 8.8 Hz), 6.11(1H,d,J-1.0 Hz), and 6.88-7.55(17H,m). (Found C,67.81; H,5.48; N,4.79; S,5.48%. C₃₃H₃₂N₂O₆S requires C,67.42; H,5.47; N,4.79; S,5.58%).
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6. Compound 2(R-H); m.p. 170-171°C; $\nu_{\text{max}}(\text{KBr})$ 3700-3000, 1760, 1720 and 1680 cm^{-1} ; $\delta(\text{d}_6\text{-DMSO})$ 1.70(3H,d,J-1.2 Hz), 2.10(3H,s), 2.18-2.35(2H,m), 2.52-2.72(2H,m), 4.51(2H,ABq,J-15.0 Hz), 5.10(1H,d,J-4.3 Hz), 5.29(1H,dd,J-4.3 and 7.6 Hz), 6.38(1H,d,J-1.2 Hz), 6.92-7.00(3H,m), 7.27-7.35(2H,m), and 9.12(1H,d,J-7.6 Hz). (Found C,57.30; H,5.26; N,6.52; S,7.60%. C₂₀H₂₂N₂O₆S requires C,57.42; H,5.26; N,6.70; S,7.66%).

7. Compound 3(R-CHPh₂); m.p. 175-177°C; $\nu_{\max}(\text{KBr})$ 1770, 1730, 1700 and 1680 cm^{-1} ; $\delta(\text{CDCl}_3)$ 1.85(3H,d,J=1.0 Hz), 2.14(3H,s), 2.49-3.10(4H,m), 4.52(3H,m), 5.84(1H,dd,J=4.8 and 10.6 Hz), 6.71(1H,d,J=1.0 Hz), 6.91-7.04(4H,m), 7.22-7.42 (12H,m) and 8.21(1H,d,J=10.6 Hz). (Found C,66.13; H,5.29; N,4.61; S,5.15%). $\text{C}_{33}\text{H}_{32}\text{N}_2\text{O}_7\text{S}$ requires C,66.00; H,5.33; N,4.66; S,5.33%.
8. Compound 4; m.p. >300°C; $\nu_{\max}(\text{KBr})$ 1761, 1703 and 1689 cm^{-1} ; $\delta(\text{CDCl}_3)$ 1.85(3H,s), 2.18(3H,s), 2.68-3.03(4H,m), 3.29(2H,ABq,J=18.0 Hz), 4.41(1H,dd,J=4.6 and 1.2 Hz), 4.58(2H,s), 6.01(1H,dd,J=4.6 and 10.4 Hz), 6.92-7.06(3H,m), 7.62-7.35(2H,m), and 7.96(1H,d,J=10.4 Hz). (Found C,58.99; H,5.59; N,7.08; S,8.17%). $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_5\text{S}$ requires C,58.46; H,5.64; N,7.18; S,8.21%.
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11. Compound 5; m.p. 160-162°C; $\nu_{\max}(\text{KBr})$ 1760, 1700 and 1670 cm^{-1} ; $\delta(\text{CDCl}_3)$ 1.80(3H,s), 2.17(3H,s), 2.64-2.87(4H,m), 3.22(2H,ABq,J=17.3 Hz), 4.57(2H,s), 4.94(1H,d,J=4.8 Hz), 5.77(1H,dd,J=10.5 and 4.8 Hz), 6.92-7.06(3H,m) and 7.24-7.36(3H,m). (Found C,60.61; H,6.22; N,7.51; S,8.41%). $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_4\text{S}$ requires C,60.94; H,5.92; N,7.48; S,8.56%.
12. Conditions normally used to de-protect trichloroethyl esters. R.B. Woodward, K.J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan and H. Vorbruggen. *J. Am. Chem. Soc.*, 1966, **88**, 852.
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14. Compound 8(R-CHPh₂; R³-CH(CO₂CH₃)CH₂CO₂CH₃); m.p. 86-90°C; $\nu_{\max}(\text{KBr})$ 1773, 1740 and 1692 cm^{-1} ; $\delta(\text{CDCl}_3)$ 1.78(3H,d,J=1.0 Hz), 2.25(1H,dd,J=17 and 1.6 Hz), 3.02(1H,dd,J=17 and 9.4 Hz), 3.45(3H,s), 3.75(3H,s), 4.38(1H,dd,J=9.4 and 1.6 Hz), 4.54(2H,s), 5.51(1H,d,J=4.6 Hz), 5.57(1H,dd,J=9.1 and 4.6 Hz), 6.20(1H,d,J=1.0 Hz), 6.90-7.10 (4H,m) and 7.20-7.42(13H,m). (Found C,63.34; H,5.14; N,4.17; S,4.49%). $\text{C}_{35}\text{H}_{34}\text{N}_2\text{O}_9\text{S}$ requires C,63.81; H,5.20; N,4.25; S,4.89%.

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